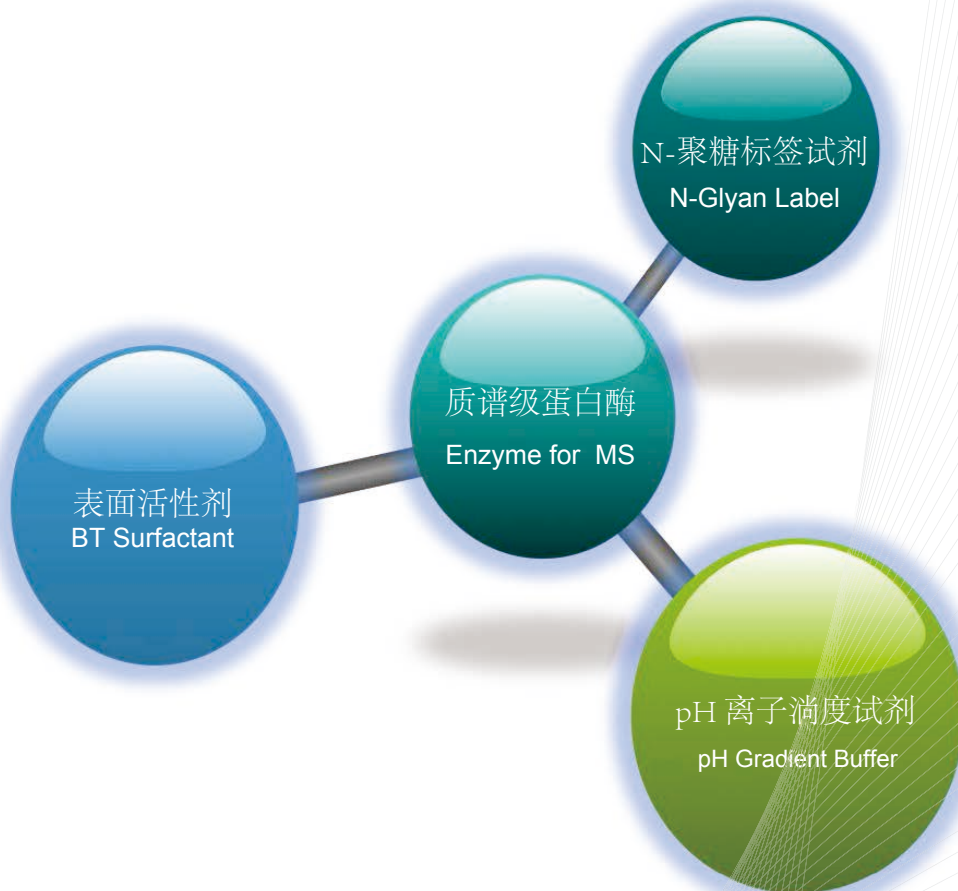


 **Endoproteinase provider** for Biopharma

Reagents Manual for Life Science



Products Quality:

Best Activity and Repeatability  
For R&D only  
LC-MS Test Report  
Technology Package  
Logistics by Dry Ice Package



## Product Information | Certification of Analysis

### I Product Information

CAS: 9002-07-7

Lot No.

#### Trypsin, Mass Spec Grade

Part No.	Name	Size/pkg
HLS TRY001C	Trypsin, Mass Spec Grade	100 µg
HLS HCL001	1 mM hydrochloric acid buffer	0.5 mL

**Description:** Trypsin specifically hydrolyzes peptide bonds at the carboxyl side of lysine and arginine residues. Unmodified trypsin is subject to auto-proteolysis, generating fragments that can interfere with protein sequencing or HPLC peptide analysis. In addition, autoproteolysis can result in the generation of pseudo trypsin, which has been shown to exhibit chymotrypsin-like specificity. Mass Spec Grade modified trypsin is porcine trypsin modified by reductive methylation, rendering it resistant to proteolysis digestion. In enzymatic stability tests, modified trypsin was not found to self-hydrolyze but can retain higher the activity of general trypsin.

**Physical Appearance:** Lyophilized powder.

**Molecular Weight:** 23 kDa

**Resuspension Buffer (HLS CHL001C):** Trypsin Resuspension Buffer is composed of 1mM hydrochloric acid.

**Storage Conditions:** Store the lyophilized powder at -20 °C; store reconstituted enzyme at -80 °C.

**Shelf life:** 24 months at -20 °C

**Stability:** Modified trypsin is maximally active in the pH range of 7-9 and reversibly inactivated at pH 4.

#### Usage Notes:

- For maximum activity, resuspend trypsin in the resuspension buffer provided, and heat at 30 °C for 15 min before use.
- Thaw the reconstituted trypsin at room temperature, placing on ice immediately after thawing.
- Add trypsin to a protease : protein ratio to be in range of 1:100 - 1:25 (w/w) for protein identification (recommended). Mix well and incubated for 37 °C for 4 h.

### I Quality Control

**Purity:** > 99.5% trypsin peak area analyzed by HPLC at 280 nm.

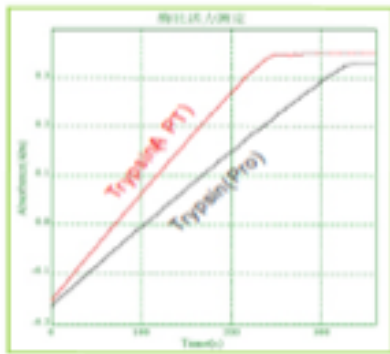
**Specificity:** < 5% nonspecific cleavage with Human Serum Albumin (HSA) sample. Digested products that were incubated at 37 °C for 16 h were compared with those incubated at 37 °C for 1 h, and the nonspecific cleavage was analyzed by LC-MS/MS.

**Activity:** 13,397 units/mg

Unit Definition: One unit is the amount of Mass spectrum Grade Modified Trypsin required to produce a  $\Delta A_{253}$  of 0.001 per minute at 37°C with the substrate N $\alpha$ -benzoyl-L-arginine ethyl ester (BAEE).

**MALDI-TOF Analysis:** Trypsin is analyzed by MALDI-TOF, impurity peak is not found.

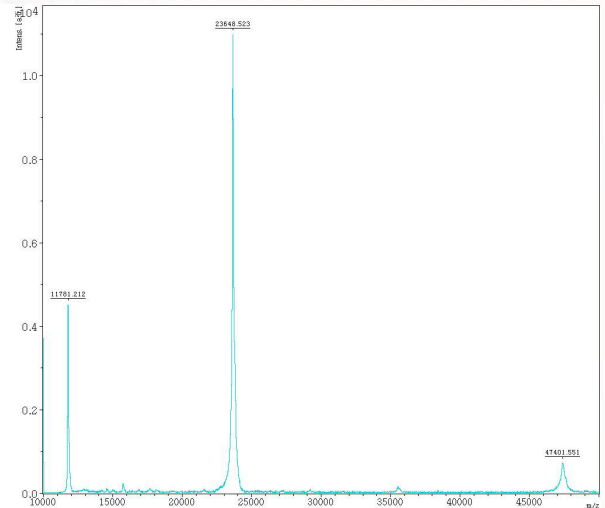
**LC-MS/MS Analysis:** HSA was dissolved and denatured for at 37 °C for 1 h, diluted at pH 8.0, and incubated with trypsin for 4 h. The digests were analyzed by LC-MS/MS, and experimental peptides results matched the peptides generated in a theoretical digestion results of HSA by trypsin.



$\text{U/mg protein} = \frac{\Delta \text{Absorbance} \times 2000 \times 3}{540 \times \text{mg Trypsin} \times \text{min}}$   
 \* 1000 μg/ml + 4.0 ml 底物 + 1.0 ml 酶液 + 1.0 ml 反应液 + 2.0 ml 水

样品	斜率 ( $\Delta A247/\text{g}$ )	TAME (units/mg protein)	BAE (units/mg protein)
Trypsin (pro)	K=0.001548	172	9890.0
Trypsin (apt-01)	K=0.002098	233	13397.5

方法：分光光度计时间扫描  
 (条件：底物为TAME, 247nm, 25℃, 6min)



1 MEQVTFISLL FIFSSAYSRG VFRDRAHKSE VAHRPFDLGE ENFKALVLIA  
 51 FAQYLQCCPF EDHVKLVNEV TEFARTCVAD ESAENCCKSL HTLFGDKLCT  
 101 VATLRETYGE MADCCARQEP ERNECFLQHK DDNPMLPRLV RPEVDVMCTA  
 151 FHDNEETFLK KYLYEIAARR FYFYAPELLF FAKRYKAAPT ECCQAADKAA  
 201 CLLPKLDELRL DEGRKASSAQ RLKCASLQKF GERAFKAMAV ARLSQRFFKA  
 251 EPAEVSKLVT DLTRVHTGCC HGDLLCADD RADLAKYICE NQDSISSRLK  
 301 ECCEKPLEK SHCIAEVEND EMPADLPSLA ADFVESKDVV KNYAEAKDVF  
 351 LGMFLYFYAR RHPDYSVLL LRLAKTYETT LERCCAAADP HECYARVFE  
 401 FKPLVEEPQN LIRQNCELFE QLGEYKQNA LLVRYTKVVP QVSTPTLVEV  
 451 SRNLGRVGSK CCKHPEAGRM PCAEDYLSVV LNQLCVLHEK IPVSDRVTEC  
 501 CTESLVNRRP CFSALEVDET YVFKEFNAET FTFHADICTL SEKERQIKKQ  
 551 TALVELVKHK FKATKEQLKA VMDDFAAPVE KCKKADDRKET CFAEBSGKGLV  
 601 AASQAALGL

QA Manager Signature:

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## Product Information | Certification of Analysis

### Product Information

CAS: 9002-07-7

Lot No.

### Trypsin 3.0, Mass Spec Grade

Part No.	Name	Size/pkg
HLS rTRY001C	rTrypsin 3.0, Mass Spec Grade	20 $\mu$ g

**Description:** rTrypsin 3.0 is a recombinant protease that specifically hydrolyzes peptide bonds at the carboxyl side of lysine and arginine residues. Engineered with a redesigned gene sequence and methylated lysines, it offers ultra-fast protein hydrolysis, high activity, and no self-degradation. rTrypsin 3.0 is ideal for protein characterization, single-cell proteomics, and large cohort proteomics studies.

**Physical Appearance:** Lyophilized powder with 20 $\mu$ g Trehalose

**Molecular Weight:** 23.7kDa

**Resuspension Buffer(HLS HAC001C):** Dissolved in 40  $\mu$ L of 50 mmol acetic acid, concentration 0.5  $\mu$ g/ $\mu$ L

**Storage Conditions:** Store the lyophilized powder at  $-20^{\circ}\text{C}$ ; store reconstituted enzyme at  $-80^{\circ}\text{C}$

**Shelf life:** 24 months at  $-20^{\circ}\text{C}$

**Stability:** rTrypsin 3.0 is maximally active in the pH range of 7–9

#### Usage Notes:

1. Suitable enzyme digestion buffers: 20 mmol Tris, 50 mmol ABC, or HEPES buffer; pH 7-9.
2. Use a 1:50 protease-to-protein ratio with a protein concentration of 0.5  $\mu$ g/ $\mu$ L. Incubate at  $37^{\circ}\text{C}$  in a dry bath for 30 minutes.

### Quality Control

**Purity:** > 99.0% based on trypsin peak area, analyzed by HPLC at 280 nm.

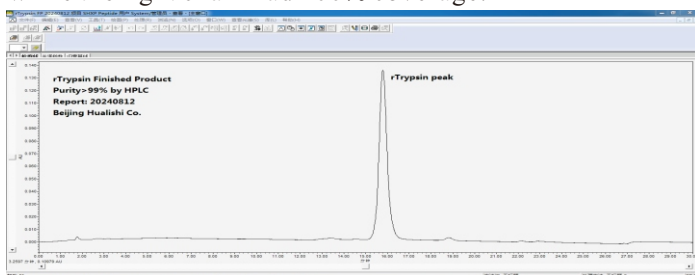
**Activity:** rTrypsin: 15,000 units/mg.

**Unit Definition:** At pH 7.8 and  $25^{\circ}\text{C}$ , 1.0  $\mu$  mol of BAEE protein is hydrolyzed per minute at 253 nm.

**Specificity:** Secukinumab antibody protein sample analyzed by ESI-MS/MS, showing > 99.8% specificity at K and R cleavage sites.

#### LC-MS/MS Analysis:

Secukinumab antibody protein was denatured, reduced, and alkylated with BT57 reagent at  $60^{\circ}\text{C}$ , pH 8.0, followed by incubation at  $45^{\circ}\text{C}$  for 30 minutes and HPLC-MS/MS analysis. The heavy chain showed 70% coverage (1 missed cleavage), while the light chain had 100% coverage.



#### QA Manager Signature:

## Product Information | Certification of Analysis

### Product Information

CAS: 9002-07-7/78642-25-8  
Lot No.

#### rTrypsin/rLys C mixer, Mass Spec Grade

Part No.	Name	Size/pkg
HLS rTRYLYSC	rTrypsin/rLys C mixer, Mass Spec Grade	20µg

**Description:** The rTrypsin 3.0 and rLys C enzyme mix combines two recombinant proteases for efficient peptide bond hydrolysis. rTrypsin 3.0 cleaves at the carboxyl side of lysine and arginine residues, while rLys C targets lysine residues specifically. This combination overcomes issues with missed cleavages due to rTrypsin's slower digestion of lysine and arginine, PTM modifications on lysines, or hydrophobic C-termini (e.g., proline). The rTrypsin and rLys C mix is ideal for protein characterization, single-cell proteomics, and large cohort proteomics studies.

**Physical Appearance:** Lyophilized powder with 67 µg Trehalose.

**Molecular Weight:** rTrypsin 23.7kDa; rLys C 27kDa.

**Resuspension Buffer (HLS HAC001C):** Dissolved in 40 µL of 50 mmol acetic acid, yielding a concentration of 0.5 µg/µL.

**Storage Conditions:** Lyophilized powder: -20°C; Reconstituted enzyme: -80°C

**Shelf life:** 24 months at -20 °C.

**Stability:** rTrypsin 3.0/rLys C is maximally active in the pH range of 7–9 .

#### Usage Notes:

1. Suitable digestion buffers: 20 mmol Tris, 50 mmol ABC, or HEPES buffer (pH 7–9).

2. Use denatured protein samples at a 1:50 protease-to-protein ratio. Maintain a protein concentration of 0.5 µg/µL and incubate at 37°C for 30 minutes.

### Quality Control

**Purity:** > 99.0% based on trypsin peak area, analyzed by HPLC at 280 nm.

**Specificity:** Secukinumab antibody protein sample analyzed by ESI-MS/MS, showing > 99.8% specificity at K and R cleavage sites.

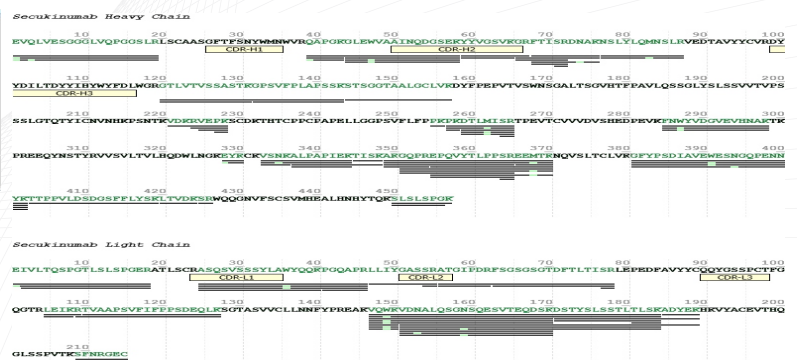
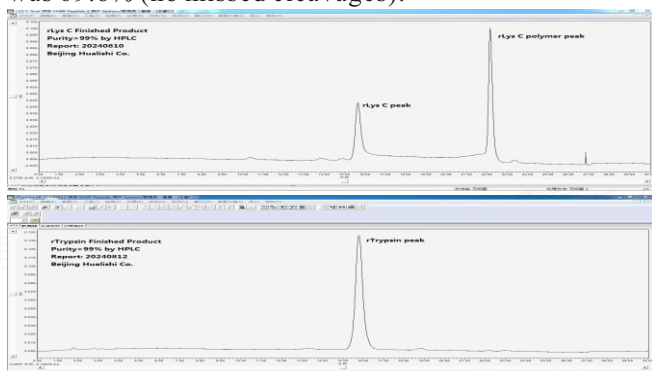
#### Activity:

· **rTrypsin:** 15,000 units/mg. Unit Definition: 1.0 µmol BAAE hydrolyzed per minute at pH 7.8, 25°C, 253 nm.

· **rLys C:** 25 units/mg. Unit Definition: 1.0 µmol T-G-P Lys Nitroanilid hydrolyzed per minute at pH 7.8, 25°C, 405 nm.

#### LC-MS/MS Analysis:

Secukinumab antibody was denatured, reduced, and alkylated using BT57 reagent at 60°C, pH 8.0, incubated at 37°C for 30 minutes, and analyzed by HPLC-MS/MS. Heavy chain coverage was 55% (no missed cleavages), and light chain coverage was 69.8% (no missed cleavages).



#### QA Manager Signature:

## Product Information | Certification of Analysis

### I Product Information

CAS: 78642-25-8

Lot No.

#### rLys-C I Mass Spec Grade

Part No.	Name	Size /pkg
HLS LYS001C	rLys-C, Mass Spec Grade	20 µg
HLS HAc001	50 mM Acetic acid buffer	0.5 mL

**Description:** rLys-C, Mass Spec Grade, specifically hydrolyzes peptide bonds at the carboxyl side of lysine residues. 8 M urea solution is recommended for denaturation. rLys-C can efficiently cleave difficult-to-digest proteins because rLys-C remains its activity under denaturation conditions.

**Physical Appearance:** Lyophilized powder

**Molecular Weight:** 27 kDa

**Resuspension Buffer (HLS HAc001C):** rLys C Resuspension Buffer is composed of 50 mM Acetic acid buffer.

**Storage Conditions:** Store the lyophilized powder at -20 °C; store reconstituted enzyme at -80 °C.

**Shelf life:** 24 months at -20 °C

**Stability:** rLys-C is maximally active in the pH 8 and reversibly inactivated at pH 3.

#### Standard In-Solution Protein Digestion:

1. For maximum activity, resuspend Mass Spectrum Grade rLys-C in resuspension buffer provided.
2. Recommend using 6 - 8 M urea and 50 mM Tris-HCl (pH 8) to denature protein or protein mixture prior to digestion.
3. Add rLys-C to the mixture to reach a protein : protease ratio of 25 : 1 (w/w). Mix well and incubate at 37 °C for 3 - 4 hours.

### I Quality Control

**Purity:** > 99.9% rLys-C peak area analyzed by HPLC at 280nm.

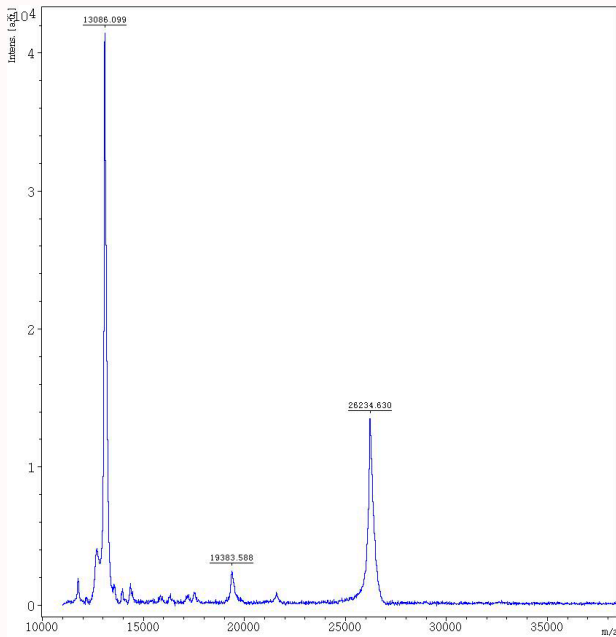
**Specificity:** < 5% nonspecific cleavage of *Escherichia Coli* digests with incubation at 37 °C for 4 h, analyzed by LC-MS/MS.

**Activity:** 25 units/min/mg

**Unit Definition:** One unit is the amount of Mass spectrum Grade rLys-C will hydrolyze 1.0 µmol of N-p-Tosyl-Gly-Pro-Lysp-Nitroanilide per minute at PH=7.7 at 25°C, A405, Light path=1cm.

**MALDI-TOF Analysis:** rLys-C is analyzed by MALDI-TOF, impurity peak is not found.

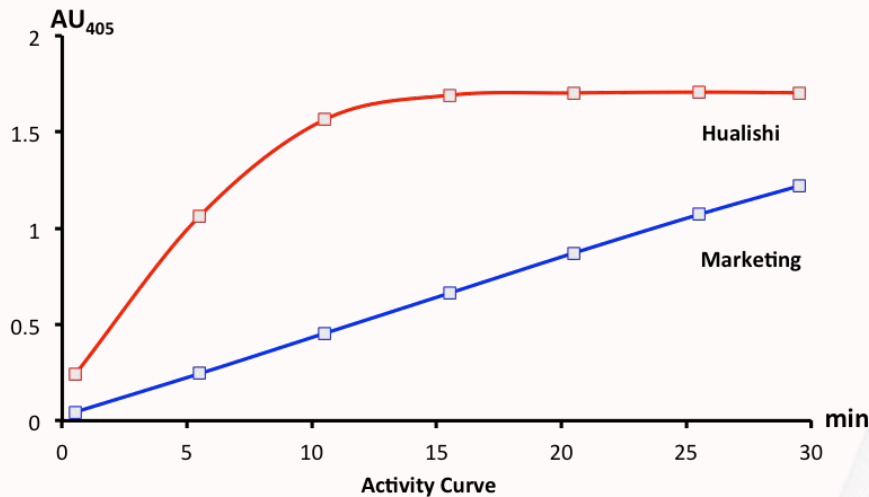
**LC-MS/MS Analysis:** Human Serum Albumin (HSA) was dissolved, denatured at 37°C for 1 h, diluted at pH 8.0, and incubated with rLys-C for 4 h. The digest was analyzed by LC-MS/MS, and peptide results matched the peptides generated in a theoretical digests of HSA by rLys-C.



Sample:	E.coli	rLys-C-1	rLys-C_2
Total of MS spectra		19128	18663
Peptide spectra match		3918	3902
MS2 identified (%)		20.48	20.91
Searched peptide		2650	2589
specific cleavage peptide(K) ratio		2531	2474
		95.51	95.56

```

1  MKWVTFISLL  FLPSSAYSRG  VFRDAKSE  VAHRFRDLGE  ENFKALVLIA
51  FAQYLQQCFP  EDHVKLVNEV  TEPARTCVAD  ESAENCCKSL  HTLFGDKLCT
101 VATLRETYGE  MADCCAKQEP  ERNECFLQHK  DDNPNLPRLV  RPEVDVMCTA
151 FHDNEETFLK  KYLYEIARRH  PYPYAFELLF  FAKRYKAAFT  ECCQAADKAA
201 CLLPKLDELK  DEGRASSAKQ  RLFCASLQKF  GERAFKAVAV  ARLSQRPFKA
251 EPAEVSRLVT  DLTRVHTECC  HGDLLCADD  RADLAKYICE  NQDSISSKLE
301 ECCEKPLLEK  SHCIAEVEND  EMPADLPSLA  ADPVESKQVC  KNYAEAKDVF
351 LGMFLYFYAR  RHPDYSVLL  LRLAKTYETT  LERCCAAADP  HECYAKVFDE
401 FKPLVEEPQN  LIRQNCLEFE  QLGEYKQNA  LLVRYTEKVP  QVSTPTLVEV
451 SRNLGRVGSK  CCKHPKAKRM  PCAEDYLSV  LNQLCVLHEK  TPFVSDRVTK
501 CTESLVNRRP  CPSALEVDET  YVPKEFNAET  FTFHADICTL  SEKERQIKKQ
551 TALVELVKHK  PKATKQLKA  VMDDFAAFVE  KCCFADRET  CFAEBGKLV
601 AASQAALGL
  
```



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## Product Information | Certification of Analysis

### I Product Information

CAS: 66676-43-5

Lot No.

#### rGlu-C I Mass Spec Grade

Part No.	Name	Size/pkg
HLS GLU001C	rGlu-C, Mass Spec Grade	50µg

**Description:** Endoproteinase rGlu-C, Mass Spec Grade, is a serine proteinase that specifically cleaves peptide bonds at C-terminal to glutamic acid residues. Enzyme specificity to glutamic acid is high in ammonium bicarbonate and ammonium acetate. In phosphate buffers, cleavage occurs at both aspartic and glutamic residues. rGlu-C activity is optimal in the pH range of 4.0 – 9.0. This mass spec grade enzyme can be used alone or in combination with other proteases to generate protein digests.

**Physical form:** Lyophilized in the presence of trehalose (1.65mg/vial)

**Molecular Weight:** 24 kDa

**Resuspension Buffer:** Resuspend rGlu-C powder in double-distilled water from user.

**Storage Conditions:** Store the lyophilized powder at  $-4\text{ }^{\circ}\text{C}$  or  $20\text{ }^{\circ}\text{C}$ ; store reconstituted enzyme solution at  $-20\text{ }^{\circ}\text{C}$  for up to 8 weeks.

**Shelf life:** 36 months at  $-20\text{ }^{\circ}\text{C}$

**Stability:** Maximally active in the pH range of 4-9

#### In-Solution Protein Digestion Protocol (recommended):

1. For maximum activity, resuspend Mass Spectrum Grade rGlu-C with the double-distilled from user.
2. Dissolve protein samples with Tris-HCl (pH 8) buffer to a final protein concentration of 50 mM prior to digestion.
3. Add rGlu-C proteinase to a protein : proteinase ratio of 20 : 1(w/w). Mix well and incubate at  $37\text{ }^{\circ}\text{C}$  for 4 h.

### I Quality Control

**Purity:**  $> 99.5\%$  peak area of rGlu-C, analyzed by HPLC at 280 nm.

**Specificity :** No prominent nonspecific peaks are observed by HPLC analysis after 16 hours of incubation of HSA with Glu-C compared to the 1-hour digestion

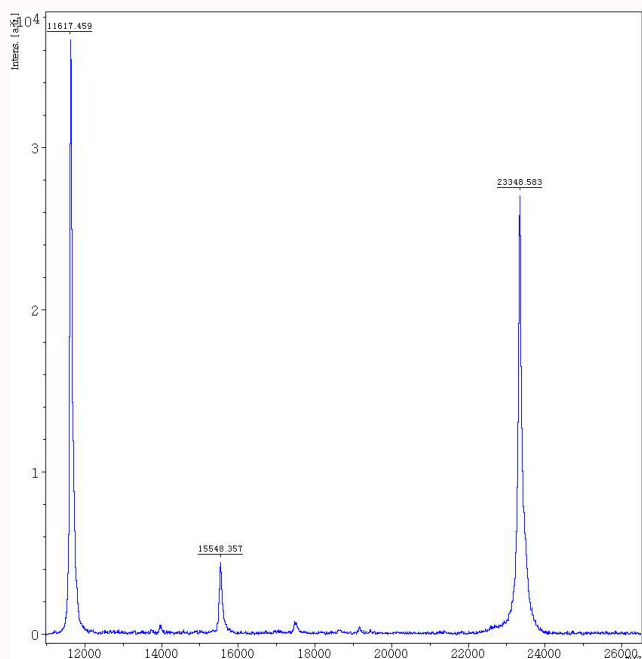
**Activity:** 800 U/min/mg.

**Unit Definition:** One unit is the amount of Mass spectrum Grade rGlu-C will hydrolyze 1.0  $\mu\text{mol}$  of N-t-Boc-L-glutamic acid  $\alpha$ -phenyl ester per minute at pH 7.8 at  $37\text{ }^{\circ}\text{C}$ , A270, and Light path=1 cm

**MALDI-TOF Analysis:** Impurity is not found in rGlu-C proteinase analyzed by MALDI-TOF.

**LC-MS/MS Analysis:** Human Serum Albumin (HSA) was dissolved, denatured at  $37\text{ }^{\circ}\text{C}$  for 1 h, diluted at pH 8.0, and incubated with rGlu-C for 4 h. The digest was analyzed by LC-MS/MS, and peptide results matched the peptides generated in a theoretical digests of HSA by rGlu-C.





## {MATRIX} Mascot Search Results

### Protein View

Match to: D6RHD5 Score: 11617  
Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=1  
Found in search of HSA-TEST-GLU-C\_20151119-mgf.mgf

Nominal mass ( $M_r$ ): 53565; Calculated pI value: 6.45  
NCBI BLAST search of [D6RHD5](#) against nr  
Unformatted [sequence string](#) for pasting into other applications

Fixed modifications: Carbamidomethyl (C)  
Variable modifications: Acetyl (N-term), Oxidation (M)  
Cleavage by V8-DE: cuts C-term side of BDEZ unless next residue is P  
Sequence Coverage: 82%

Matched peptides shown in **Bold Red**

```

1  MSQLKIVTNIH LYLVEIARRH PFFYAPPELLF FAKRYKAAFT ECCQAADKAA
51 CLLPRLDELR DEGRASSAKQ RLRCSLQKPF GERAPKAWAV ARLSQRFPKA
101 EFAEVSKLVT DLTKVHTECC HGDLLCADD RADLAKYICE NQDSISSKLE
151 ECCEKPLLEK SHCIAEVEND EMPADLPSLA ADPVESKDVC KNYAEARDVP
201 LGMFLYEYAR RHPDYSVLL LRLAKTYETT LEKCCAAADP HECYARVFDE
251 FKPLVEEPQN LIKQNCLEPF QLOEYKFNQA LLVRYTKKVP QVSIPTLVEV
301 SRNLGKVGSK CCKHPEAKRM PCAEDYLSVV LNQLCVLHEK TPVSDRVTEK
351 CTESLVNRRP CFSALEVDET YVPKEFNAET FTFHADICTL SEKERQIKKQ
401 TALVELVGHK PKATKQQLKA VMDDFAAFVE KCKKADDDKET CFAEEGKGLV
451 AASQAALGL

```

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## Product Information | Certification of Analysis

### I Product Information

CAS: 83534-39-8

Lot No.

#### rPNGase F I Mass Spec Grade

Part No.	Name	Size /pkg
HLS PNG001C	rPNGase F, Mass Spec Grade	50 $\mu$ L

**Description:** PNGase F (Peptide-N-glycosidase F) is a protease for deglycosylation of glycoproteins, and is produced by recombinant protein expression. The enzyme hydrolyze the N-terminal of the asparagine (Asn) residue side chain to release asparagine-linked oligosaccharides from glycoproteins and glycopeptides. The oligosaccharides can be high mannose, hybrid, or complex type.

**Molecular Weight:** 35 kDa

**Composition:** rPNGase F in 20 mM Tris-HCl (pH 7.5 at 25 °C), 50 mM NaCl, and 5 mM EDTA solution

**Concentration:** 10 units/ $\mu$ L

**Storage Conditions:** Store at 2–8 °C.

**Shelf life:** 12 months at 2–8 °C

**Stability:** rPNGase F is maximally active in the pH range of 6-10, best at pH 8.6 .

#### In-Solution Protein Digestion Protocol:

1. For maximum activity, resuspend the user's samples (eg. glycoprotein) with 50 mM ammonium bicarbonate buffer (pH 7.8).
2. Dissolve 20  $\mu$ g samples in 50 mM ammonium bicarbonate (pH 7.8) to a final volume of 18  $\mu$ L.
3. Add 2  $\mu$ L rPNGase F to the solution in step 2.. Mix well and incubate at 37 °C for 30 min.

### I Quality Control

**Purity:** > 99.0% rPNGase F peak area analyzed by HPLC at 280 nm.

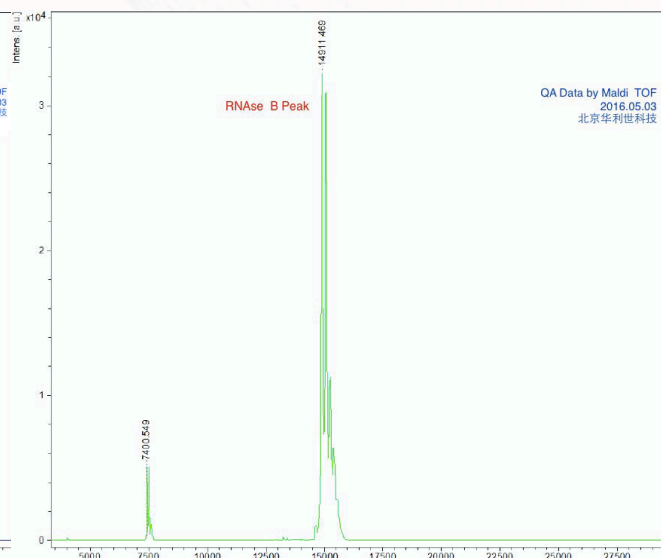
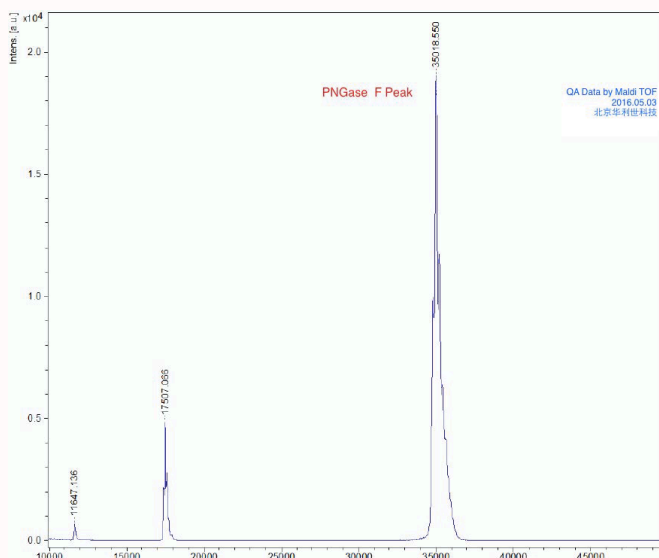
**Specificity:** Denatured RNase B (50  $\mu$ g) is incubated with rPNGase F at 37 °C for 60 min, and analyzed by SDS-PAGE. Deglycosylation is assessed by the presence of deglycosylated RNase B.

**Activity:** 40,000 units/min/mg

**Unit Definition:** One unit catalyzes the release of N-linked oligosaccharides from 1 nanomole of denatured Ribonuclease B in 1 min at 37 °C at pH 7.5 monitored by SDS-PAGE.

**MALDI-TOF Analysis:** rPNGase F is analyzed by MALDI-TOF, and impurity peak was not found.

**LC-MS/MS Analysis:** Denatured RNase B was incubated with rPNGase F for 2 h to release glycans and cleaved by the trypsin enzyme. The digested peptides were analyzed by LC-MS/MS, from which nine peptides in the experimental results matched the peptides generated in a theoretical digestion result.



**Protein Identification Details**

Coverage: ProteinCard

Ribonuclease pancreatic OS-Bos taurus GN-RNASE1 PE-1 SV-1 -[RNAS1\_BOVIN]

Annotate PTMs reported in Uniprot  
 Show only PTMs  
 Include PSMs that are filtered Out

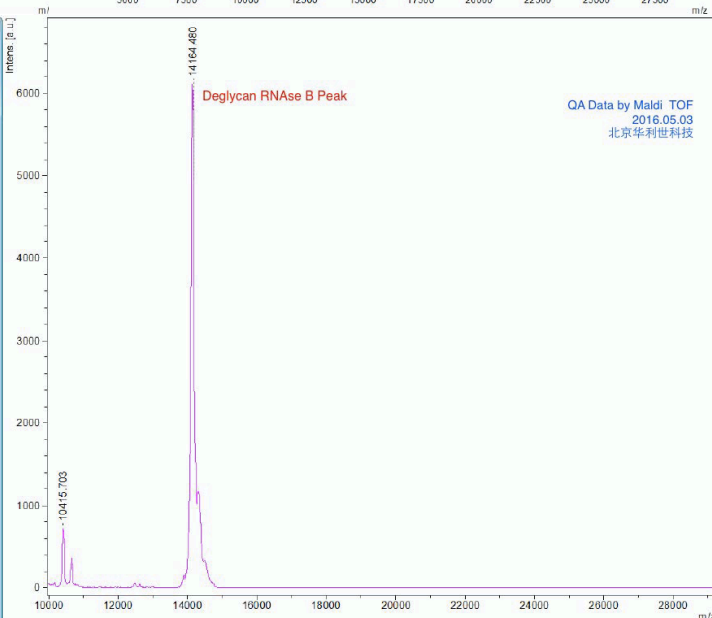
Coverage: 67.33%

**Found Modifications:**

- C Carbamidomethyl (C)
- O Oxidation (M)

Sequence	Modification List
1 11 21 31 41 51 61 71 81 91 101 111 121 131 141 150	
1 MALKSLVLLS LVLVLLVLR VQPSLGGKTA AAKFED <b>GRD</b> SSTSAASSN	
51 C O O C C C C C C C C C C C C C C	
YCH <b>GRD</b> RRSN LTKDRCKPVN FVIRSLAVV QAVCSQNVVA C <b>GRD</b> TRCYD	
101 O C C C C C C C C C C C C C C C C	
SYSTNSITDC RRYCCKYPN CAVKTRQANK IILVACEQNP YVPHEDASV	
151	

OK Help



QA Manager Signature:

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## Product Information | Certification of Analysis

### I Product Information

CAS: EC 3.4.24.20

#### Lys-N | Mass Spec Grade

Lot No.

Part No.	Name	Size /pkg
HLS LYS001N	Lys-N, Mass Spec Grade	20 µg
HLS HAc001Zn	50 µM Zinc Acetic buffer	200 µL

**Description:** Lys-N is an innovative protease that specifically hydrolyzes peptide bonds at the N-terminal of lysine residues. This enzyme can be applied for post-translational modification (PTM) protein research, and it produces more b ions than y ions in the spectrometer. Combining the use of Lys-N with rTrypsin-N gives the best way for protein sequencing.

**Physical Appearance:** Lyophilized powder

**Molecular Weight:** 18.4 kDa

**Resuspension Buffer (HLS HAc001Zn):** 50 µM Zinc Acetic buffer or Zinc Sulfate buffer.

**Storage Conditions:** Store the lyophilized powder at -20°C. Store reconstituted enzyme at -80 °C for up to 30 days.

**Shelf life:** 24 months at -80 °C.

**Stability:** Maximally active in the pH range 7 - 9.

#### In-Solution Protein Digestion Protocol:

1. Resuspend 20 µg of Mass Spectrum Grade Lys-N in 40 µL resuspension buffer for maximum activity.
2. Add 50 mM ammonium bicarbonate or Tris-HCl (pH 8) to protein mixture (recommended).
3. Add 0.5 µg/µL Lys-N to reach a final enzyme to substrate ratio of 1:30 to digest the samples. Mix well and incubate at 37 °C for 4 hours.

### I Quality Control

**Purity:** > 99.5% peak area analyzed by HPLC at 280 nm.

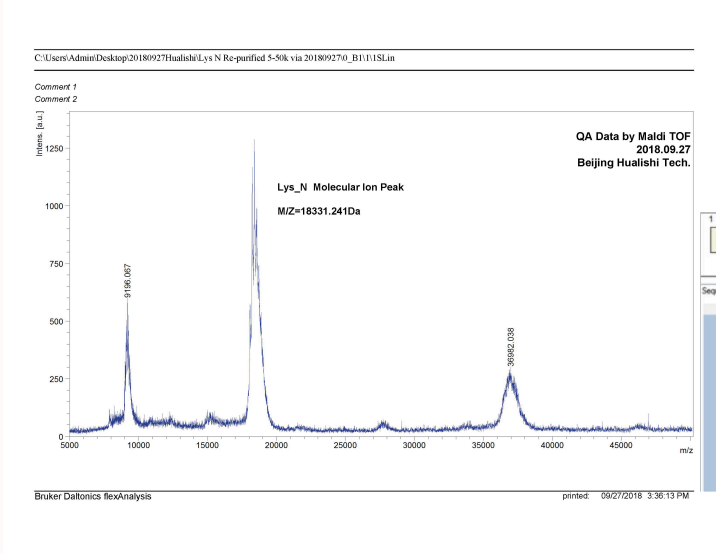
**Specificity:** < 5% nonspecific cleavage with *Escherichia Coli* digests (digestion at 37 °C for 4 hours), analyzed by LC-MS/MS.

**Activity:** 394 U/mg.

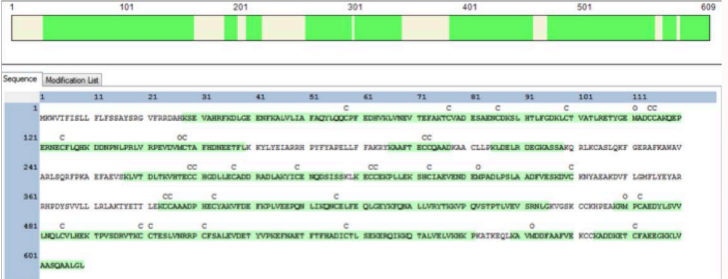
Unit Definition: 1 unit of proteolytic activity towards azocasein is defined as the amount of enzyme required for half-maximal OD366 after a 30 min incubation at 37 °C, pH 10, A366, and light path=1cm.

**MALDI-TOF Analysis:** No impurity peak found of Lys-N, analyzed by MALDI-TOF

**LC-MS/MS Analysis:** Human serum albumin (HSA) was dissolved, denatured at 37°C for 1 h, diluted at pH 8.0, and incubated with Lys-N for 4 hours. The digest was analyzed by LC-MS/MS. Experimental peptide results match the peptides generated in a theoretical digest of HSA by Lys-N.



Sample E. coli	rLys-N_Hualishi	rLys-N_Marketing
Total of Proteins	878	904
peptide	3535	3557
specific cleavage peptide(K)	3041	3020
Ratio(%)	86.00	85.00



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## Product Information | Certification of Analysis

### I Product Information

CAS: N/A

Lot No.

#### rTrypsin-N, Mass Spec Grade

Part No.	Name	Size/pkg
HLS TRY001N	rTrypsin-N, Mass Spec Grade	20 µg

**Description:** rTrypsin-N, Mass Spec Grade, is an innovative protease that specifically hydrolyzes peptide bonds at the amino side of lysine and arginine residues. rTrypsin-N can be applied for post-translational modification (PTM) proteins research and is best for protein sequencing. Peptides produced more b ions than y ions in the mass spectrometer.

**Physical Appearance:** Supplied by lyophilized powder with 0.47 mg HEPES; 0.05mg CaCl<sub>2</sub> per vial.

**Molecular Weight:** 29 kDa

**Resuspension Buffer:** 200 µL distilled water, pH 7.5

**Storage Conditions:** Store the lyophilized powder at -20°C, and reconstituted enzyme at -80 °C for up to 30 days.

**Shelf life:** 24 months at -80 °C

**Stability:** rTrypsin-N is maximally active at pH 7.5.

#### In-Solution Protein Digestion Protocol:

1. For maximum activity, resuspend Mass Spec Grade rTrypsin-N in 200 µL distilled water, pH 7.5.
2. Use 20 - 50 mM HEPES solution, 0.1% BT Surfactant, or Tris-HCl (pH 7.5) for the protein mixture cleavage (recommended).
3. Add 0.5µg/µL rTrypsin-N to a final enzyme : substrate ratio of 1 : 30. Mix well and incubated at 37 °C for 4 h.

### I Quality Control

**Purity:** > 99.5% rTrypsin-N peak area analyzed by HPLC at 280 nm.

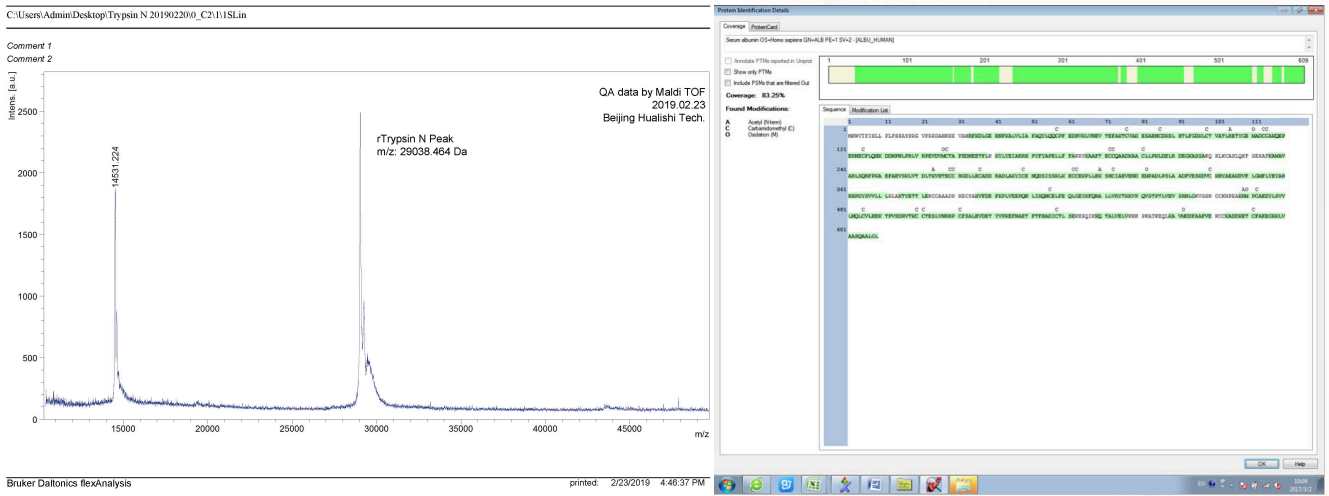
**Specificity:** < 5% nonspecific cleavage with Human Serum Albumin (HSA) sample. Digested products were incubated at 37 °C for 4 h, and nonspecific cleavage was analyzed by LC-MS/MS.

**Activity:** 152 units/mg

Unit Definition: One unit is the amount of Mass spectrum Grade rTrypsin-N will hydrolyze per minutes at pH 10 at 25 °C, OD366, Light path = 1 cm.

**MALDI-TOF Analysis:** rTrypsin-N is analyzed by MALDI-TOF, impurity peak is not found.

**LC-MS/MS Analysis:** HSA samples were dissolved and denatured for at 37 °C for 1 h. The denatured HSA was diluted at pH 7.5 and incubated with rTrypsin-N for 4 h. The digest was analyzed by LC-MS/MS, and the peptides results matched the peptides generated in a theoretical digestion results of HSA by rTrypsin-N.



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## Product Information | Certification of Analysis

### | Product Information

#### $\alpha$ -Chymotrypsin | Mass Spec Grade

Part No.	Name	Size
HLS CHY001C	$\alpha$ -Chymotrypsin	50 $\mu$ g

CAS: EC 3.421.1

Lot No.

**Description:** Chymotrypsin is a serine endoprotease that specifically cleaves the C-terminal peptide bonds of Tyr, Phe, Trp, and Leu. It also cleaves some C-terminal peptide bonds of Met, Ala, Asp, and Glu. The residual trypsin can be inactivated by TLCK and purified. The protease can be activated and stabilized by  $\text{Ca}^{2+}$  ions.

**Physical Appearance:** Lyophilized powder.

**Molecular Weight:** 25 kDa.

**Resuspension Buffer:** Reconstitute lyophilized powder in 100  $\mu$ L 1 mM hydrochloric acid containing 2  $\mu$ M  $\text{CaCl}_2$  (recommended).

**Storage Conditions:** Store freeze-dried powder at  $-20\text{ }^\circ\text{C}$  refrigerator; store dissolved enzyme at  $-20\text{ }^\circ\text{C}$ , valid for 1 week.

**Shelf Life:** 24 months at  $-80\text{ }^\circ\text{C}$ .

**Stability:** Maximally active in the pH range of 7.8.

**In-Solution Protein Digestion Protocol:** Use  $\alpha$ -chymotrypsin at a protease to protein ratio of 1: 50 (w/w), and perform digestion in 100 mM Tris-HCl (pH 7.8) containing 10 mM  $\text{CaCl}_2$  at  $30\text{ }^\circ\text{C}$  for 2 - 12 hours.

#### Tips:

1. The protease will self-hydrolyze if the water bath temperature is higher than  $37\text{ }^\circ\text{C}$ .
2. Preferably  $< 1\text{M}$  urea or guanidine hydrochloride concentration during the sample digestion.

### | Quality Control

**Purity:**  $> 99.0\%$  peak area analyzed by HPLC at 280 nm.

**Specificity:** For human serum albumin samples,  $> 95.0\%$  specificity of ESI-MS/MS for Y, F, W and L terminus.

**Activity:** 70 U/mg. Unit Definition: The amount of 1.0  $\mu$ mol BTEE protein hydrolyzed by chymotrypsin per minute at pH 7.8 at  $25\text{ }^\circ\text{C}$ .

**MALDI-TOF Analysis:** No impurity protein peaks were found in the purified protease using matrix laser desorption mass spectrometry.

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## Product Information | Certification of Analysis

### | Product Information

**CAS: 9001-62-1**  
**Lot No.**

#### Lipase | Mass Spec Grade

Part No.	Name	Size
HLS LIP001C	Lipase	50 µg

**Description:** Lipase catalyzes the hydrolysis of triacylglycerols in aqueous solutions to generate glycerol and free fatty acids. The lipase is derived from porcine pancreas.

**Physical Appearance:** Lyophilized powder.

**Molecular Weight:** 48 kDa

**Resuspension Buffer:** Dissolve with 100 µL of 5 mM calcium chloride (ice bath before use).

**Storage Conditions:** Powder at -20 °C.

**Shelf Life:** 12 months at -20 °C

**pH Range:** Maximally active at pH 7.7.

**Application:** Lipase specifically hydrolyzes triacylglycerols in serum or animal tissue samples

#### **In-Solution Protein Digestion Protocol (Recommended):**

Add 20 µL of 100 mM PBS buffer into 20 µL serum sample and adjust to pH 7.7. Incubate samples at 37 °C for 30 min. Then, immediately add 2 - 6 µL lipase and incubate for 15min.

### | Quality Control

**Purity:** > 99.0% peak area measured by HPLC at 280 nm. Impurity protein peak was detected.

**Activity:** 20000 U/mg.

**Unit Definition:** The amount of enzyme required to hydrolyze triglycerides to release 1.0 microequivalents of fatty acids in 1 hour with olive oil as a substrate at pH 7.7 and a temperature of 37 °C.

**LC-MS/MS Analysis:** N/A

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## Product Information | Certification of Analysis

### I Product Information

CAS: 916777-57-6

Lot No.

#### rldeS I Mass Spec Grade

Part No.	Name	Size
HLS IDES001	rldeS	20 µg
HLS PBS001	digestion buffer	0.5 mL

**Description:** rldeS, mass spec grade, is an engineered recombinant protease overexpressed in *Escherichia coli*. It cleaves specifically at a single recognition site below the hinge region to produce homogenous F(ab')<sub>2</sub> and Fc fragments. Both IdeS and IdeZ proteases effectively cleave human IgG1, IgG2, IgG3, and IgG4, monkey, sheep, rabbit, humanized and chimeric IgG, and Fc fusion proteins. However, only the IdeZ protease cleaves mouse IgG2a and IgG3.

**Physical Appearance:** Lyophilized powder

**Molecular Weight:** 37 kDa

**Resuspension Buffer (reference):** 40 µL double-distilled water from user

**Digestion Buffer:** 10X 50 mM PBS, 150 mM NaCl (pH 6.6) solution

**Storage Conditions:** Store lyophilized powder at -20 °C.

**Shelf Life:** 24 months at -20 °C

**pH Range:** Maximally active at pH 6-8

**Application:** rldeS protease is an IgG-specific degradation enzyme to produce homogeneous F(ab')<sub>2</sub> and Fc fragments.

#### In-Solution Protein Digestion Protocol (Recommended):

- Mix 5 µL 10X enzyme digestion buffer (HLS PBS001) and double-distilled water.
- Add 10 µL 10 µg/µL mAb samples to the mixture.
- Add 4 µL 0.5 µg/µL rldeS (HLS IDE001) to the mixture to total reaction volume of 50 µL.
- Incubate at 37 °C for 30 min.

### I Quality Control

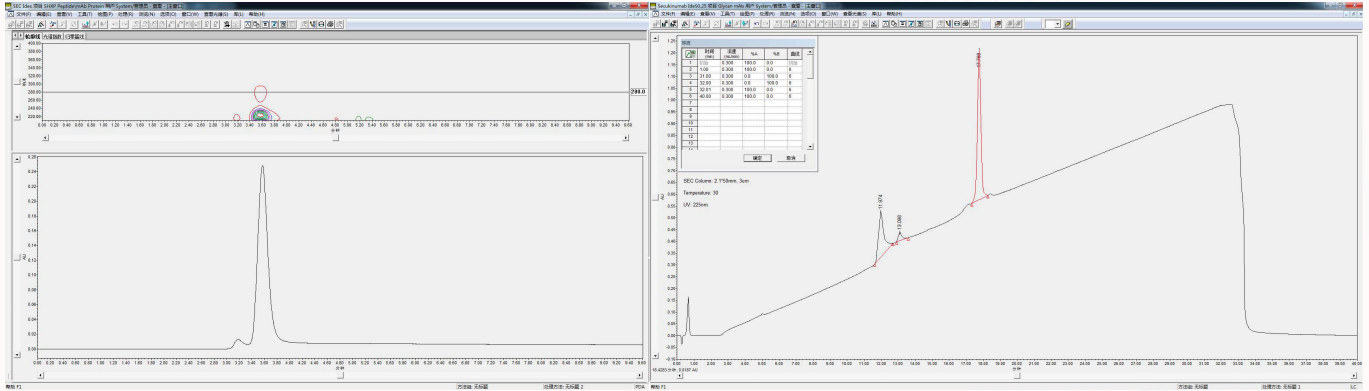
**Purity:** > 99.5%

**Specificity:** > 99.3% purity of mAb antibody samples after rldeS digestion and elution with mAB pH ion mobility buffer, analyzed by UPLC chromatography with SCX 2.1\*50 mm column.

**Activity:** 50 units/µg

Unit Definition: One unit is the amount of 1 µg IdeS required to produce 50 µg substrate IgG antibody at 37 °C in 30 min.

**MALDI-TOF Analysis:** Purified rldeS was analyzed by HCCA matrix laser analytical mass spectrometry. No impurity peak was found.



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## Product Information | Certification of Analysis

### I Product Information

CAS: 916777-57-6

Lot No.

#### rldeZ I Mass Spec Grade

Part No.	Name	Size
HLS IDEZ001	rldeZ	20 µg
HLS PBS001	digestion buffer	0.5 mL

**Description:** rldeZ, MS grade, is an engineered recombinant protease overexpressed in *Escherichia coli*. It cleaves specifically at a single recognition site below the hinge region to produce homogenous F(ab')<sub>2</sub> and Fc fragments. Both rldeS and rldeZ proteases effectively cleave human IgG1, IgG2, IgG3, and IgG4, monkey, sheep, rabbit, humanized and chimeric IgG, and Fc fusion proteins. However, only the rldeZ protease cleaves mouse IgG2a and IgG3.

**Physical Appearance:** Lyophilized powder

**Molecular Weight:** 37 kDa

**Resuspension Buffer (reference):** 40 µL double-distilled water from user

**Digestion Buffer:** 10X 50 mM PBS, 150 mM NaCl (pH 6.6) solution

**Storage Conditions:** Powder at -20 °C

**Shelf Life:** 24 months at -20 °C

**pH Range:** Maximally active at pH 6-8

**Application:** rldeS protease is an IgG-specific degradation enzyme to produce homogeneous F(ab')<sub>2</sub> and Fc fragments.

#### In-Solution Protein Digestion Protocol (Recommended):

- Mix 5 µL 10X enzyme digestion buffer (HLS PBS001) and double-distilled water.
- Add 10 µL 10 µg/µL mAb samples to the mixture.
- Add 4 µL 0.5 µg/µL rldeS (HLS IDE001) to the mixture to total reaction volume of 50 µL.
- Incubate at 37 °C for 30 min.

### I Quality Control

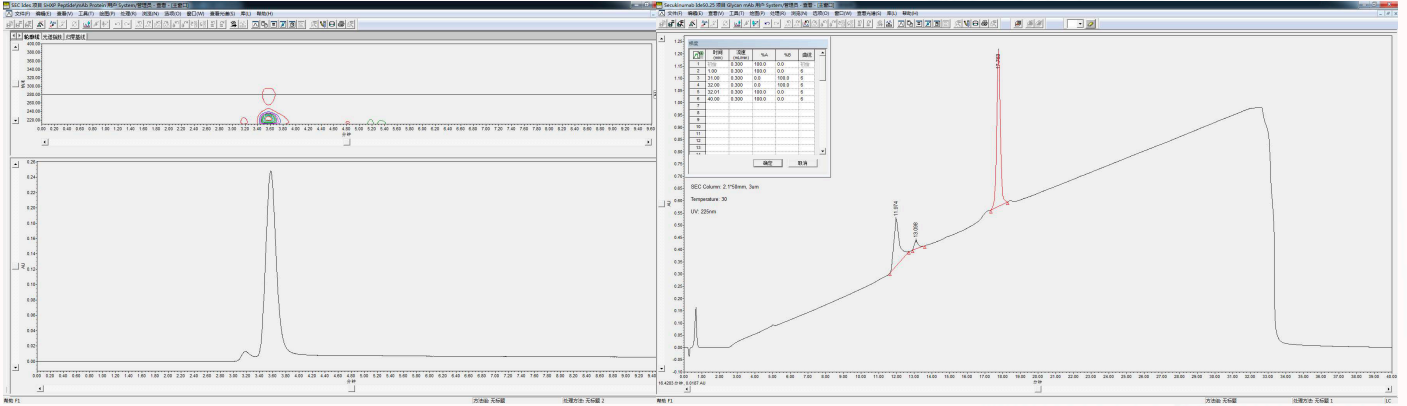
**Purity:** > 99.5%

**Specificity:** > 99.3% purity of mAb antibody samples after rldeS digestion and elution with mAB pH ion mobility buffer, analyzed by UPLC chromatography with SCX 2.1\*50 mm column.

**Activity:** 50 units/µg

Unit Definition: One unit is the amount of 1 µg rldeZ required to produce 50 µg substrate IgG antibody at 37 °C in 30 min.

**MALDI-TOF Analysis:** Purified rldeZ was analyzed by HCCA matrix laser analytical mass spectrometry. No impurity peak was found.



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## Product Information | Certification of Analysis

### I Product Information

CAS: N/A  
Lot No.

#### mAb Standard I Mass Spec Grade

Part No.	Name	Size
Secukinumab	mAb Standard	100 µg

**Description:** Secukinumab monoclonal antibody is an interleukin-17A (IL-17A) inhibitor, which can be used for intact protein quality control (QC) and charge variants (CV). Also, it assists with the mAb subunit and N-glycan LC-MS analysis, purification, and system suitability verification of experimental methods.

**Physical Appearance:** Mass spectrometry modified trypsin is supplied by lyophilized powder.

**Molecular Weight:** 147940.0 Da

**Molecular Formula:** C<sub>6584</sub>H<sub>10134</sub>N<sub>1754</sub>O<sub>2042</sub>S<sub>44</sub>

**Resuspension Buffer:** Add a certain amount of pH Buffer to dissolve according to the experimental needs.

**Storage Conditions:** Store the lyophilized powder at -20°C.

**Shelf Life:** 24 months at -20 °C.

**Amino Acid Sequence:**

#### > Secukinumab Heavy Chain (CAS 875356-43-7)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMNWVRQAPGKGLEWVAAINQDGSEKYYVGSVKGRFTISRDN  
NAKNSLYLQMNSLRVEDTAVYYCVRDYYDILTDYYIHYYWYFDLWGRGTLTVSSASTKGPSVFPLAPSSKSTSG-  
GTAALGCLVKDYFPEPVTVSWNSGALTSQVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVD-  
KRVEPKSCDKTHTCPPCPAPELGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK-  
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN-  
QVSLTCLVKGFIYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCVMHEALHN-  
HYTQKSLSLSPGK

#### > Secukinumab Light Chain (CAS 875356-44-8)

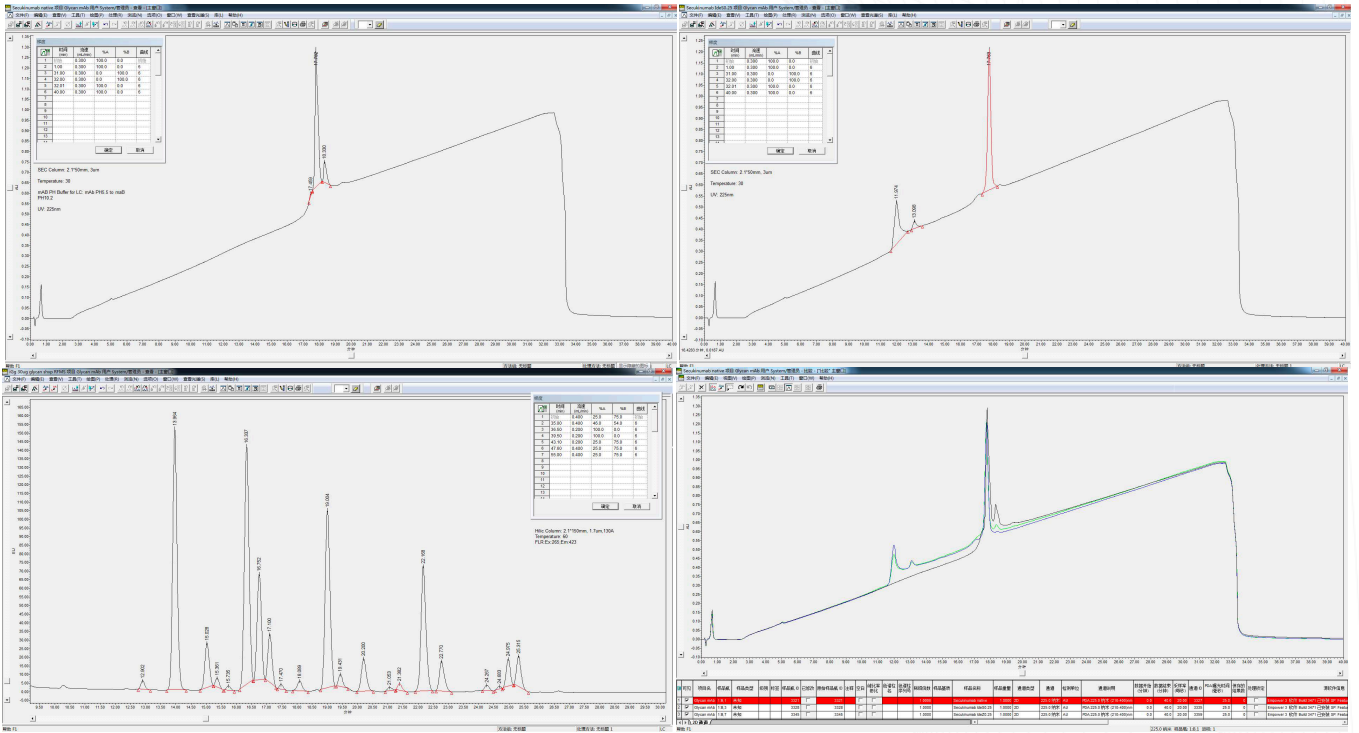
EIVLTQSPGTLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISR-  
LEPEDFAVYYCQQYGSSPCTFGQGRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD-  
NALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

### I Quality Control

**Purity:** 99.5% peak area measured by HPLC at 280 nm.

**CV and Subunit by HPLC:** The mAb antibody samples were digested with IdeS or IdeZ. Then, the consistency was analyzed by HPLC with SCX 2.1\*50 mm column at 30 °C, followed by elution with mAb PH ion mobility buffer.

**N-Glycans by HPLC:** 15 µg sample was reduced at 95°C, denatured with TCEP, and digested with rPNGase F glycosidase. N-glycans were derivatized into glycosamines with N-glycan labeling reagents. Samples were analyzed by UPLC fluorescence detection using the HILIC column (FLR: Ex: 265 nm; Em: 425 nm).



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## Product Information | Certification of Analysis

### | Product Information

**CAS: 881640-19-3**

**Lot No.**

### BT Surfactant | Mass Spec Grade

Part No.	Name	Size
HLS BTS001C	BT Surfactant	5 mg

**Description:** Protein denaturation and enzymolysis are essential experimental steps in analyzing peptides and glycans of biopharmaceutical antibodies and proteomics. The highly structural characteristics of proteins in their natural forms pose obstacles for enzymes to reach the cutting sites. In order to expose the proteins' enzymatic cutting sites and make them effectively interact with the enzymes, proteins must undergo complete denaturation, reductive alkylation, and appropriate enzymatic reaction. Common denaturants, including urea, guanidine hydrochloride, and SDS, will carry out specific chemical modifications of the protein, such as carbamylation, which may cause the error in polypeptide identification. However, the **BT Surfactant** can effectively avoid many unfavorable post-translational modifications after protein denaturation.

**Physical Appearance:** Lyophilized powder

**Molecular Weight:** 393.28 Da

**Resuspension Buffer (reference):** Resuspend 5 mg BT Surfactant powder in 167  $\mu$ L user's buffer (pH 8.0) or double-distilled water to get 3% (w/v) solution.

**Storage Conditions:** Powder at -20 °C, reconstituted solution at -80 °C

**Shelf Life:** 24 months at -80 °C as solution; long-term effective at -20 °C as powder

**pH Range:** Maximally active for enzymatic reaction at pH 7-9; degrade and precipitate at pH 2-4.

**Temperature:** < 1% BT Surfactant peak area after incubation at 37 °C for 4 hours; < 5% BT Surfactant peak are after incubation at 95 °C for 10 min.

### Reference Procedure:

1. Denature protein samples in 1-2% (w/v) **BT Surfactant** solution.
2. Digest proteins with user's protease in 0.1% (w/v) BT Surfactant solution.

### BT Surfactant's Influence on common proteases:

No observable effects on **Trypsin** activity in < 2% BT Surfactant.

No observable effects on **rLys C** activity in < 1% BT Surfactant.

No observable effects on **rPNGase F** activity in < 1% BT Surfactant.

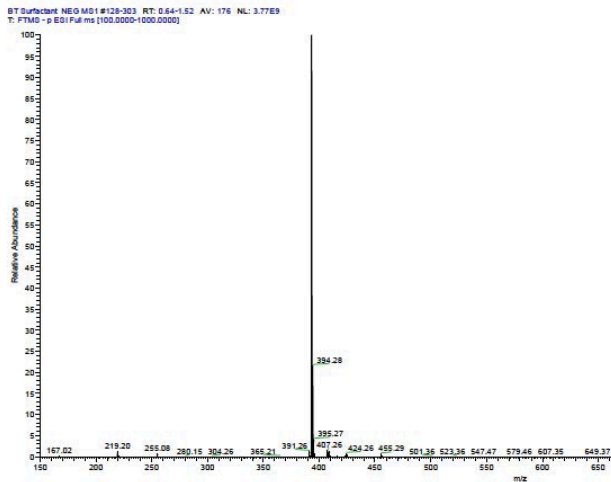


## | Quality Control

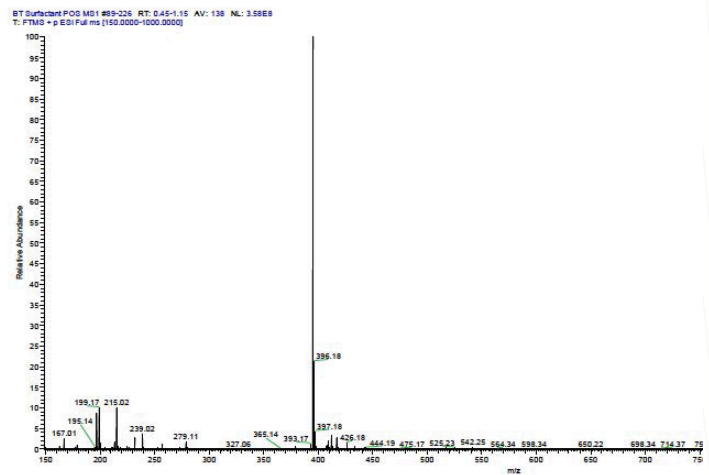
**Purity:** > 99.9% BT Surfactant peak area, analyzed by Thermo QE HF mass spectrometry coupled with positive ion mode ESI.

**Degradation:** 0.1% BT Surfactant peak area after 0.2% (v/v) formic acid incubation at 45 °C for 30 min, analyzed by QEHF mass spectrometry coupled with negative ion mode ESI.

ESI- MS1/MS2 Mass Spec. for BT Surfactant Finish Product



ESI+ MS1/MS2 Mass Spec. and LC/MS for BT Surfactant Finish Product

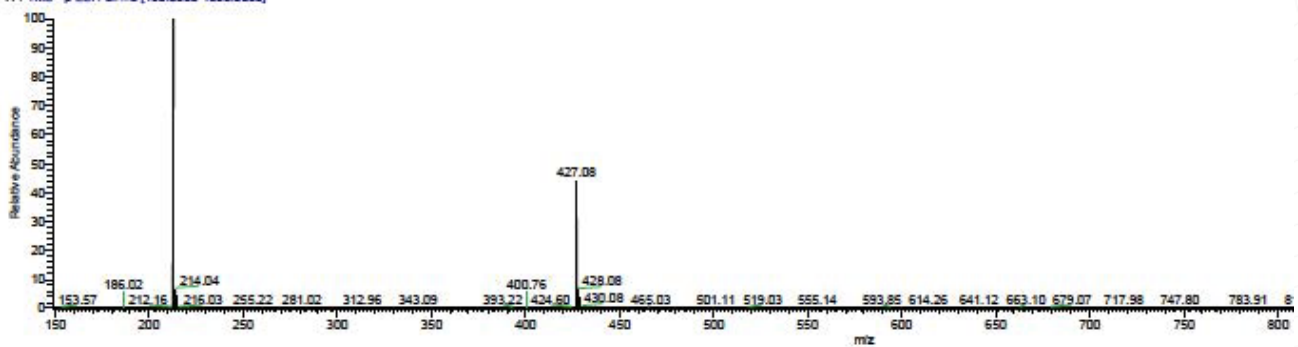


## ESI- Mass Spec. and LC/MS for BT Surfactant Finish Product by FA Degradation

BT Surfactant\_Formic Acid Degradation

12/17/20 15:18:10

BT Surfactant\_Formic Acid Degradation #121-649 RT: 2.42-2.56 AV: 29 NL: 1.60E9  
T: FTMS - p ESI Full ms [150.0000-1000.0000]



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## Product Information | Certification of Analysis

### Product Information

CAS: 881640-19-3

Lot No.

#### BT57 Reagent, Mass Spec Grade

Part No.	Name	Size/pkg
HLS BT57001	BT57 Reagent, Mass Spec Grade	1.5 mg

**Description:** BT57 is a fast, three-in-one reagent for denaturation, reduction, and alkylation, ideal for biopharmaceutical and proteomics research. In these fields, native protein structures are typically denatured, reduced, alkylated, desalted, and then prepared for enzymatic reactions. Conventional denaturants like urea and guanidine hydrochloride can chemically modify proteins (e.g., carbamylation), and traditional alkylating agents require light protection. BT57 addresses these issues, allowing for the rapid completion of denaturation, reduction, and alkylation in a single step. It is suitable for proteomics, biopharmaceuticals, and single-cell sample research.

**Physical Appearance:** Lyophilized powder.

**Molecular Weight:** 393.28 Da.

**Resuspension Buffer:** 52 µL double-distilled water dissolve.

**Storage Conditions:** Powder at -20 °C, reconstituted solution at -80 °C.

**Shelf life:** 24 months at -80 °C as a solution; long-term stable at -20 °C as powder.

**pH Range:** pH 7-9 for protein denaturation reaction, will degrade and precipitate in solution at pH 2-4.

#### Reference Protocol:

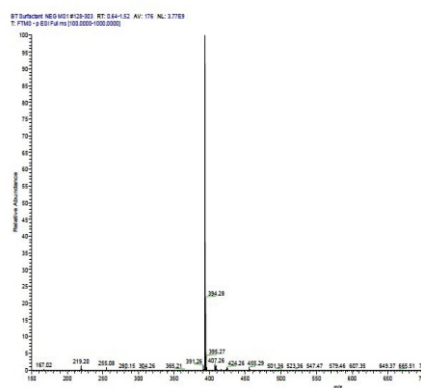
Add 10 µL BT57 to 10 µL (50 µg) protein sample, mix, and incubate at 60°C for 20 min or 95°C for 10 min.

### Quality Control

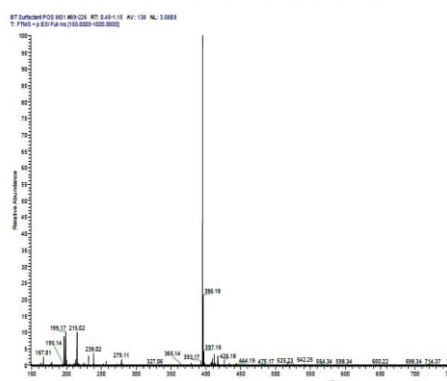
**Purity:** > 99.9% based on BT Surfactant peak area, analyzed by Thermo QE HF mass spectrometry with positive ion mode ESI.

**Degradation:** 0.1% BT Surfactant peak area after incubation with 0.2% (v/v) formic acid at 45°C for 30 min, analyzed by QE HF mass spectrometry with negative ion mode ESI.

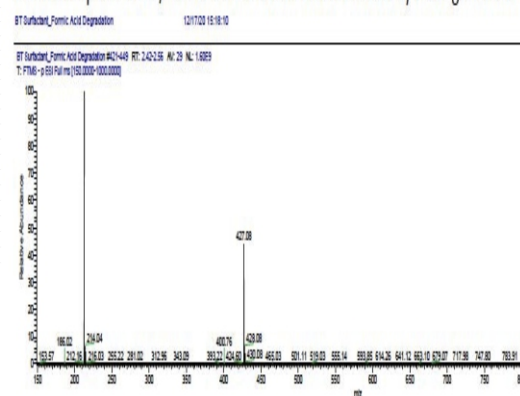
ESI- MS1/MS2 Mass Spec. for BT Surfactant Finish Product



ESI+ MS1/MS2 Mass Spec. and LC/MS for BT Surfactant Finish Product



ESI- Mass Spec. and LC/MS for BT Surfactant Finish Product by FA Degradation



QA Manager Signature:

## Product Information | Certification of Analysis

### Product Information

CAS: 881640-19-3

Lot No.

#### Digest Buffer, Mass Spec Grade

Part No.	Name	Size/pkg
HLS DIG001B	Digest Buffer, Mass Spec Grade	5 mg

**Description:** Digest Buffer is a BT surfactant-based buffer optimized for proteomics and biopharmaceutical research. It facilitates protein denaturation, reduction, and alkylation, followed by enzymatic hydrolysis to convert proteins into peptides with high efficiency and recovery. The surfactant is then degraded by formic acid, allowing direct peptide analysis via mass spectrometry without the need for desalting, minimizing peptide loss. Ideal for proteomics, biopharmaceuticals, and single-cell research.

**Physical Appearance:** Lyophilized powder with 5 mg BT Surfactant and 48 mg ammonium bicarbonate.

**Molecular Weight:** 393.28 Da.

**Resuspension Buffer:** Dissolve in 12 mL MS Grade water.

**Storage Conditions:** Powder at -20 °C, reconstituted solution at 4 °C for 6 months.

**Shelf life:** 24 months at -80 °C as a solution; long-term stable at -20 °C as powder.

**pH Range:** pH 7-9 is used for proteolysis experiments, will degrade and precipitate in solution at pH 2-4.

#### Reference Protocol:

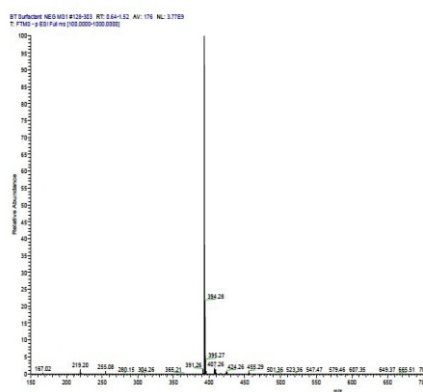
Add 100 µL Digest Buffer to 10 µL (50 µg) protein sample and mix well. Add 1 µg MS-grade protease and incubate at 42°C for 40 minutes. Then, add 3 µL formic acid and incubate at 42°C for 20 minutes.

### Quality Control

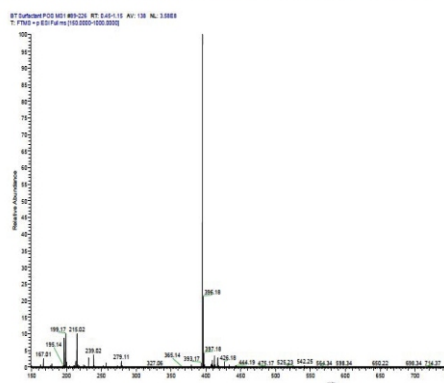
**Purity:** > 99.9% BT Surfactant peak area, analyzed by Thermo QE HF mass spectrometry coupled with positive ion mode ESI.

**Degradation:** 0.1% BT Surfactant peak area after incubation with 0.2% (v/v) formic acid at 42°C for 30 minutes, analyzed by QE HF mass spectrometry using negative ion mode ESI.

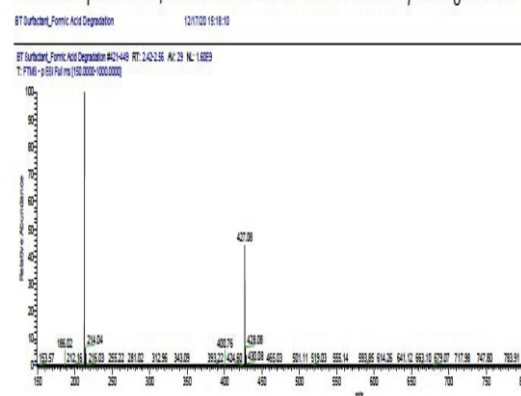
ESI- MS1/MS2 Mass Spec. for BT Surfactant Finish Product



ESI+ MS1/MS2 Mass Spec. and LC/MS for BT Surfactant Finish Product



ESI- Mass Spec. and LC/MS for BT Surfactant Finish Product by FA Degradation



QA Manager Signature:

## Product Information | Certification of Analysis

### I Product Information

CAS: 61043-41-2

#### N-Glycan Label I Mass Spec Grade

Lot No.

Part No.	Name	Size
HLS GLAB01W	N-glycan label-W	4.5 mg
HLS DMF001	Anhydrous DMF	200 $\mu$ L

**Description:** N-glycan label is used in ultra-fast labeling of glycans of PNGase F glycosidase-digested N-linked glycoproteins (including antibodies). Labeled glycan has highly sensitive fluorescence characteristics and ionization properties by mass spectrometry, allowing for quantitative analysis using LC-FLR and LC-MS. The unique chemical properties of this label provide higher fluorescence quantification and mass spectrometry response.

**Physical Appearance:** Lyophilized powder

**Molecular Weight:** 427.1850 Da

**Molecular Formula:**  $C_{21}H_{25}N_5O_5$

**Mass Shift:** +  $C_{17}H_{20}N_4O_2$  = 312.3663 Da

**Labeled N-Glycan (Da)** = Released N-Glycan + Glycan Label Reagent - Reaction by-product ( $C_4H_5NO_3$ , MW: 115.0268) - H

**Resuspension:** Dissolve 4.5 mg N-Glycan label in 55  $\mu$ L freshly prepared DMF solution to reach 82  $\mu$ g/ $\mu$ L.

**Storage Conditions:** -20  $^{\circ}$ C refrigerator as lyophilized powder.

**Shelf Life:** 24 months at -20  $^{\circ}$ C.

**Application:** 4.5 mg N-Glycan labeling reagent can label five digested glycan samples.

#### Reference Procedure:

1. Add 10  $\mu$ L desalted 1.5  $\mu$ g/ $\mu$ L mAb samples into 0.6 mL low protein binding collection tube.
2. Add 10  $\mu$ L 3% BT Surfactant with 5 mM TCEP and 50 mM HEPES (pH 7.9) to the tube and mix well.
3. Heat at 95  $^{\circ}$ C for 3 min, then cool to the room temperature.
4. Add 10  $\mu$ L 1.5 U/ $\mu$ L rPNGase F and mix well.
5. Incubated at 50  $^{\circ}$ C for 15 - 30 min, then cool to room temperature.
6. Add 10  $\mu$ L 82  $\mu$ g/ $\mu$ L Glycan Label, incubate at room temperature for 5 min.
7. Add 15  $\mu$ L anhydrous DMF and 45  $\mu$ L acetonitrile. Mix well.

Labeled N-glycan samples can be used for LC-FLR and LC-MS analysis.

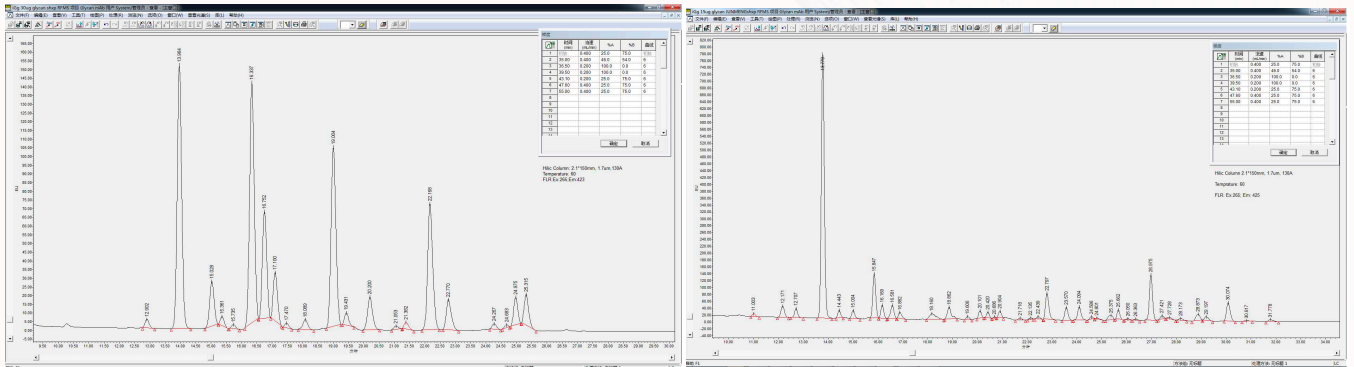
### I Quality Control

**Product Composition:** The ratio of N-Glycan Label: NHS compound is 3:2 analyzed by LC-QE HF.

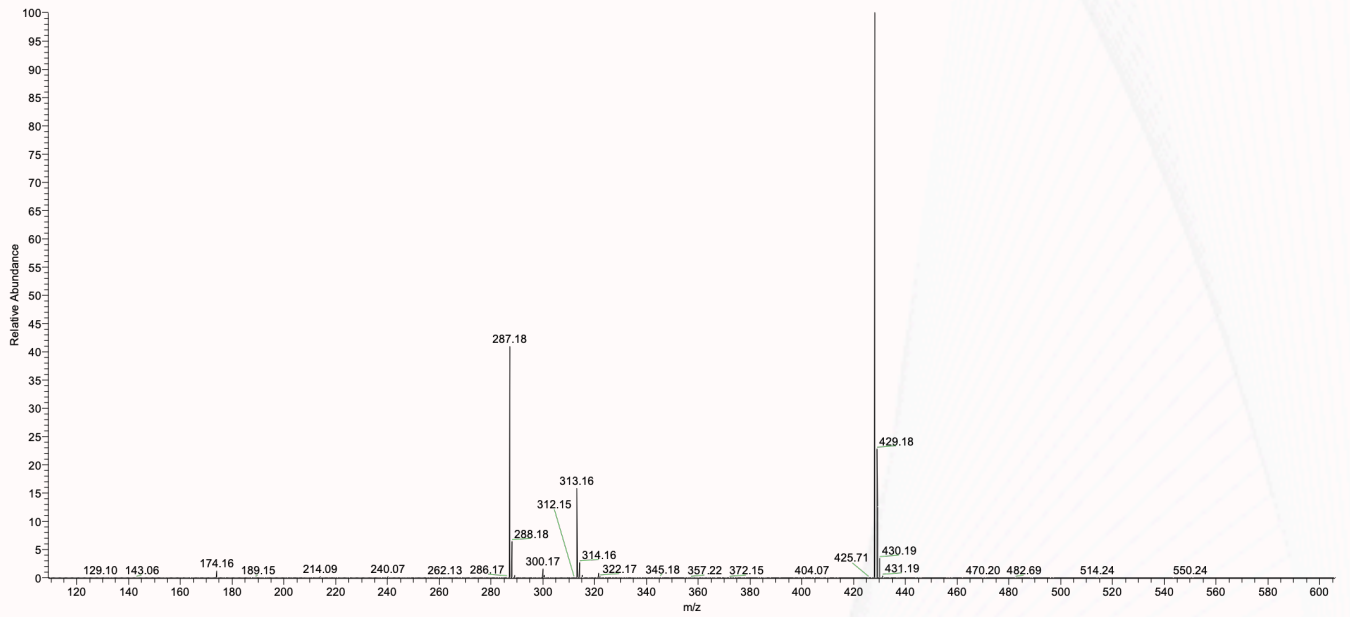
**Labeling Efficiency:** 99.5% when 0.37  $\mu$ g/ $\mu$ L mAb and 20  $\mu$ g/ $\mu$ L N-Glycan tags are used to label 15  $\mu$ g N-glycans released by glycoproteins.

**Thermal stability:** N-Glycan labeling reagent is stable in anhydrous DMF at room temperature for 8 h; Labeled glycoamines are stable at room temperature for 24 h, and can withstand 320  $^{\circ}$ C of mass spectrometry ionization temperature.

**Chemical Instability:** Unstable when concentrations of affinity reagents, SDS, Tris, DTT, amine, and mercaptans greater than 0.1 mM.



Glycan Label RFMS POS MS1 #240-420 RT: 0.69-1.21 AV: 181 NL: 4.13E9  
T: FTMS + p ESI Full ms [110.0000-600.0000]



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## Product Information | Certification of Analysis

### I Product Information

#### mAB pH Gradient Buffer I LC Buffer

CAS of Buffer A: 1266615-59-1-1132-61-2

CAS of Buffer B: 2915-38-6-73463-39-5

Lot No.

Part No.	Name	Size
HLS PH01LCA	LC pH Gradient Buffer A	6.1 g
HLS PH01LCB	LC pH Gradient Buffer B	6.65 g

**Description:** pH gradient buffer is an innovative elution buffer (or mobile phase) that changes pH linearly for separation and purification of mAb antibodies, glycoproteins and PTM modified proteins by ion exchange (IEX) chromatography. In pH gradient buffer-based IEX chromatography, the pH of the initial buffer is kept at a constant level to ensure that the charged proteins bind to oppositely charged ions of the stationary phase. Then, the protein is eluted by linearly changing the pH of the buffer so that the protein has a zero net charge and can elute from the column.

**Physical Appearance:** Lyophilized powder

**Reagent Composition:** Each bottle contains 2.5 mg BT Surfactant, 850 mg NaCl, and 6 g sulfonate.

**Resuspension:** Dissolve reagents in 500 mL deionized, double-distilled water.

**pH Adjustment:** At room temperature, use pH meter to ensure the pH error is less than 0.2, and use 2 M NaOH or hydrochloric acid to adjust the pH.

Elution Buffer (mAb): **pH Buffer A, pH 5.5**

Elution Buffer B (maB): **pH Buffer B, pH 10.2**

**Storage Conditions:** In 4 °C refrigerator as lyophilized powder.

**Shelf Life:** 24 months at 4 °C as powder; 7 days at 4 °C as solution.

**Linear pH Range:** Linear correlation coefficient > 0.99 at pH 5.5 - 10.2 in columns of IEX chromatography.

**Reference Procedure ofr mAb subunit and intact protein:**

**Column:** SCX, 10 μm, 2.1 \* 250 mm or 2.1 \* 50 mm

**Eluent A:** 1X mAb pH buffer A (pH 5.5)

**Gradient:** 0 -1 min, 0% B; 1 - 31 min, 0 - 100% B;

**Temperature:** 30 °C

**UV detection:** 280 nm

**Inject volume:** 4 μL

**Flow rate:** 0.3 mL/min

**Eluent B:** 1X maB pH Buffer B (pH 10.2)

**31 - 34 min, 100% B; 34 -40 min, 0%B**

**FLR detection:** Ex: 280 nm, Em: 360 nm

**Load:** 0.2 - 10 μg

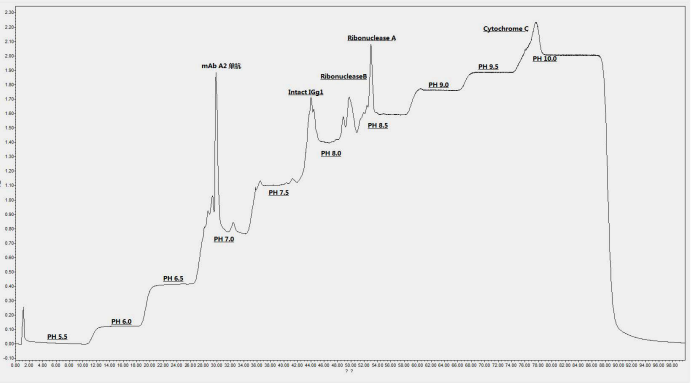
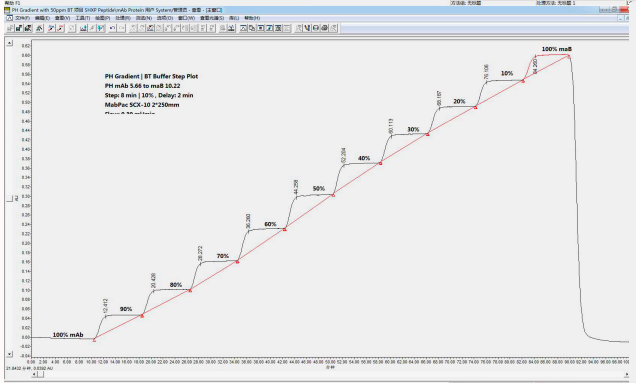
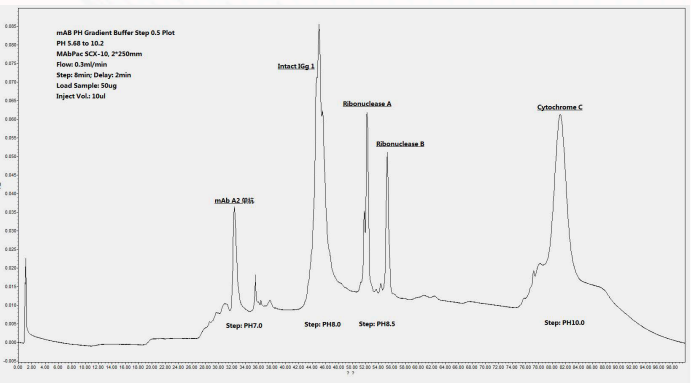
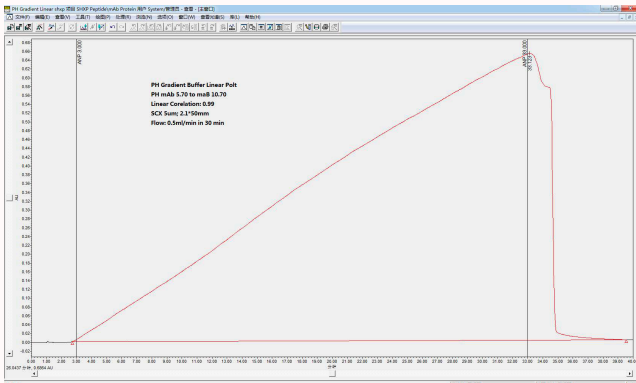
### I Quality Control

**Purity:** In ion mobility buffer, the BT Surfactant is mass spectrometry reagents, and other components are pure chromatography reagents with < 0.01 absorbance at 280 nm, analyzed by liquid chromatography.

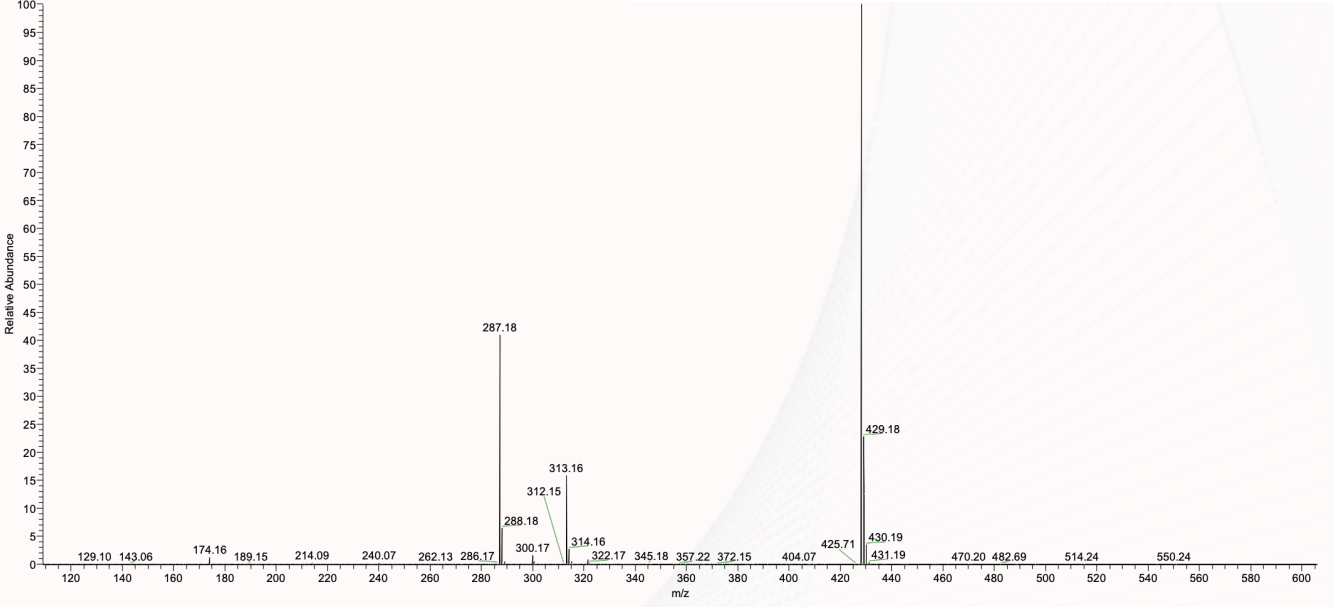
**pH Accuracy:** pH 5.5 for mAb pH Buffer A and pH 10.2 for maB pH Buffer B with error less than 0.2.

**pH Elution Linearity:** The linear correlation coefficient of 227 nm absorbance > 0.999 with zero volume injection and SCX ion exchange chromatography system in 30 min run time at 30 °C column temperature.

**pH Elution Degree of Separation:** Linear elution degree of separation > 1 with 5 pKa protein mAb: IGg A1, Ribonulease A, Ribonuclease B, and Cytochrome C.



Glycan Label RFMS POS MS1 #240-420 RT: 0.69-1.21 AV: 181 NL: 4.13E9  
 T: FTMS + p ESIFull ms [110.0000-600.0000]



QA Manager Signature:

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## Product Information | Certification of Analysis

### I Product Information

mAB pH Gradient Buffer I MS Buffer

CAS of Buffer : 6915-15-7-631-61-8

Lot No.

Part No.	Name	Size
HLS PH01MSA	MS pH Gradient Buffer A	0.60 g
HLS PH01MSB	MS pH Gradient Buffer B	1.50 g

**Description:** pH gradient buffer is an innovative elution buffer (or mobile phase) that changes pH linearly for separation and purification of mAb antibodies, glycoproteins and PTM modified proteins by ion exchange (IEX) chromatography. In pH gradient buffer-based IEX chromatography, the pH of the initial buffer is kept at a constant level to ensure that the charged proteins bind to oppositely charged ions of the stationary phase. Then, the protein is eluted by linearly changing the pH of the buffer so that the protein has a zero net charge and can elute from the column. this buffer are suitable for IEX-MS to characterize intact mAb or subunit.

**Physical Appearance:** Lyophilized powder

**Reagent Composition:** Each bottle contains 0.3-1 g Ammonium acetate, and 0.2 g Organic acid.

**Resuspension:** Dissolve reagents in 100 mL deionized, double-distilled water.

**pH Adjustment:** At room temperature, slowly add LC grade ammonia adjust pH value to ensure the Buffer A pH=5.5; the Buffer B=8.5 for ready mobile phase of LC

Elution Buffer (mAb): **pH Buffer A, pH 5.5**

Elution Buffer B (maB): **pH Buffer B, pH 8.5**

**Storage Conditions:** In 4 °C refrigerator as lyophilized powder.

**Shelf Life:** 24 months at 4 °C as powder; 1 day at 4 °C as solution.

**Linear pH Range:** Linear correlation coefficient > 0.99 at pH 5.5 - 8.5 in columns of IEX chromatography.

**Reference Procedure for mAb subunit and intact protein:**

**Column:** SCX, 10 µm, 2.1 \* 250 mm or 2.1 \* 50 mm

**Eluent A:** 1X mAb pH buffer A (pH 5.5)

**Gradient:** 0 -1 min, 0% B; 1 - 31 min, 0 - 100% B;

**Temperature:** 30 °C

**UV detection:** 280 nm

**Inject volume:** 4 µL

**Flow rate:** 0.3 mL/min

**Eluent B:** 1X maB pH Buffer B (pH 8.5)

**31 - 34 min, 100% B; 34 - 40 min, 0%B**

**FLR detection:** Ex: 280 nm, Em: 360 nm

**Load:** 0.2 - 10 µg

### I Quality Control

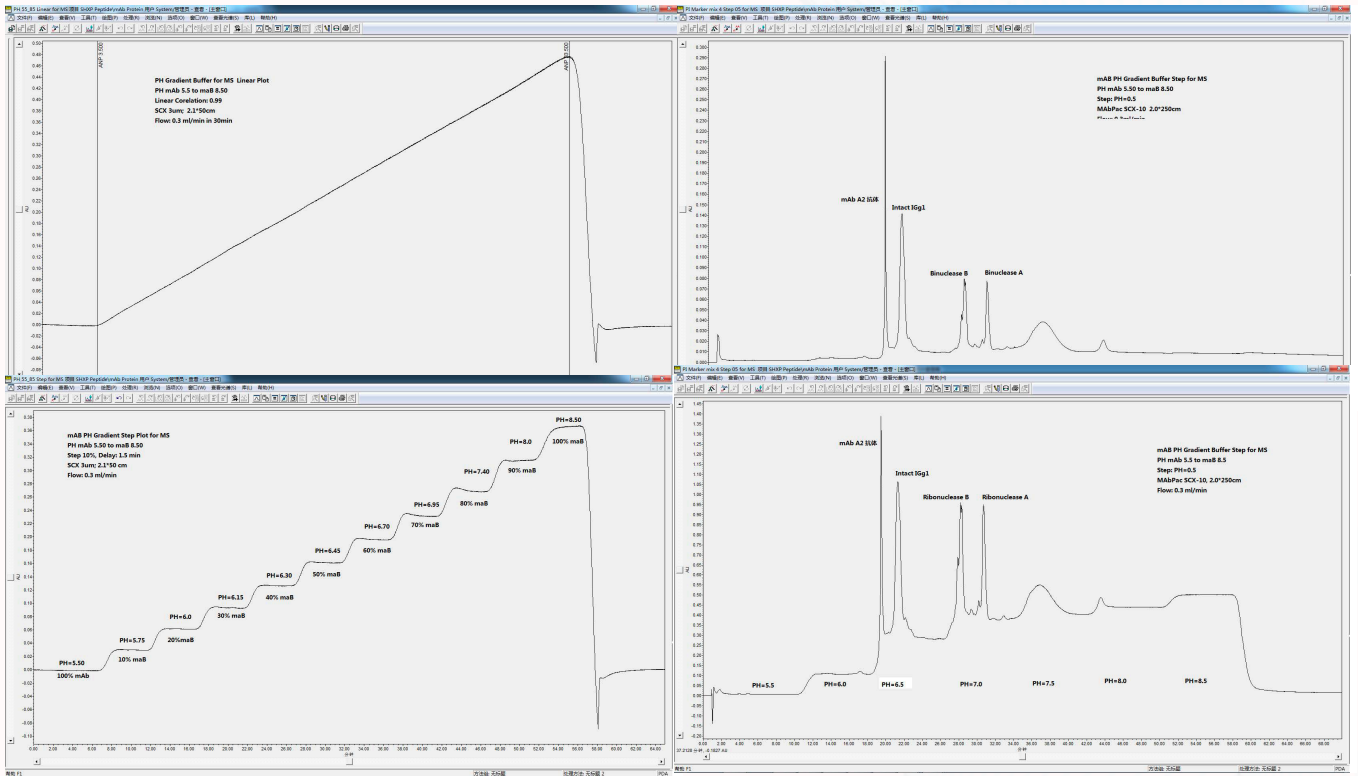
**Purity:** Pure chromatography reagents with < 0.01 absorbance at 280 m, analyzed by liquid chromatography.

**pH Accuracy:** pH 5.5 for mAb pH Buffer A and pH 8.5 for maB pH Buffer B with error less than 0.2.

**pH Elution Linearity:** The linear correlation coefficient of 225 nm absorbance > 0.999 with zero volume injection and SCX ion exchange chromatography system in 30 min run time at 30 °C column temperature.

**pH Elution Degree of Separation:** Linear elution degree of separation > 1 with 5 pKa protein mAb: IGg A1, Ribonulease A, Ribonuclease B.





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