Rapid Enzyme Digestion System
Hudson Surface Technology Inc.
visit www.maldiplate.com for more

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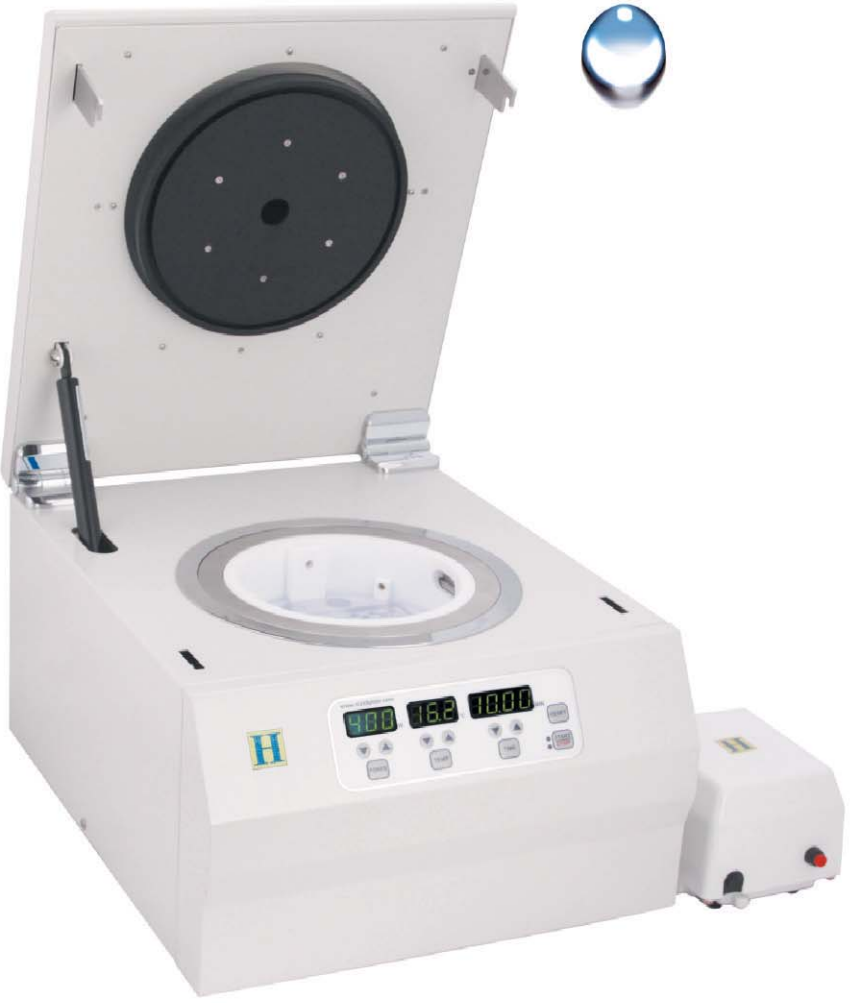
ISO 9001

ISO 14001

HST REDS

Rapid Enzyme Digestion System
Hudson Surface Technology



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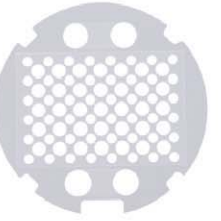
◆ Introduction

This HST Rapid Enzyme Digestion System offers low cost solutions for easy, fast and safe sample preparation. It can increases the temperature of the water in the jar as well as sample evenly and instantly by exposing the microwave onto the water molecules in the jar through transferring the electric energy of Watts into the high frequency vibration energy. This system is suitable for the sample preparation in Electrophoresis and for many enzymatic reactions such as proteolysis in the area of Proteomics, or PNGase F reaction for glycan separation from glycoproteins. This system covers not only a single sample but also 96 samples in each well of 96-well plate at one time operation for large scale protein digestion or biomarker research.

◆ Composition

LID for Water Jar (LID)



35 well 1.5 mL Tube Rack (TR)

Reaction Water Bath (JAR)

Power Cable

Air Pump

◆ Operation

1. Connection

1. Connect the Power Cable to the back of the REDS.

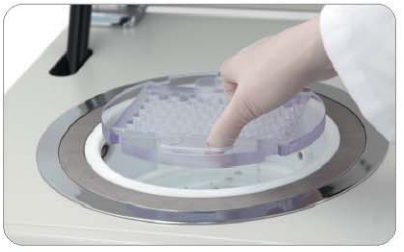
2. Connect the hose to the Air Pump.

3. Connect the other side of the hose to the back of the REDS.

4. Press the door to open it.

5. Put the Jar into the Chamber.

6. Pour the 500 mL water in the Jar.



7. Put the Tube Rack in the Jar.

8. Put the Lid on the Guide Rack.

9. Press the door to close it.

10. Unscrew the cab on the top of the door to open.

11. Press the switch located on the right side of the REDS.

12. Press the power button on the back of the Air Pump.

2. Pre-heating

1. Press the Power button.

2. Set the parameters by pressing the ▼ or ▲ buttons.

3. Press the Power button once again.

4. Set the temperature and reaction time.

5. Press the Start button.

6. When the pre-heating is finished, press the Stop button.

• Tip (Example) At 800 W, Set the temperature approximately 3 ℃ lower than the reaction temperature (around 2~3 minutes needed).

3. Setting

1. Press the Reset button and place the sample.

2. Set the power, temperature, reaction time.

3. Press the Start button.

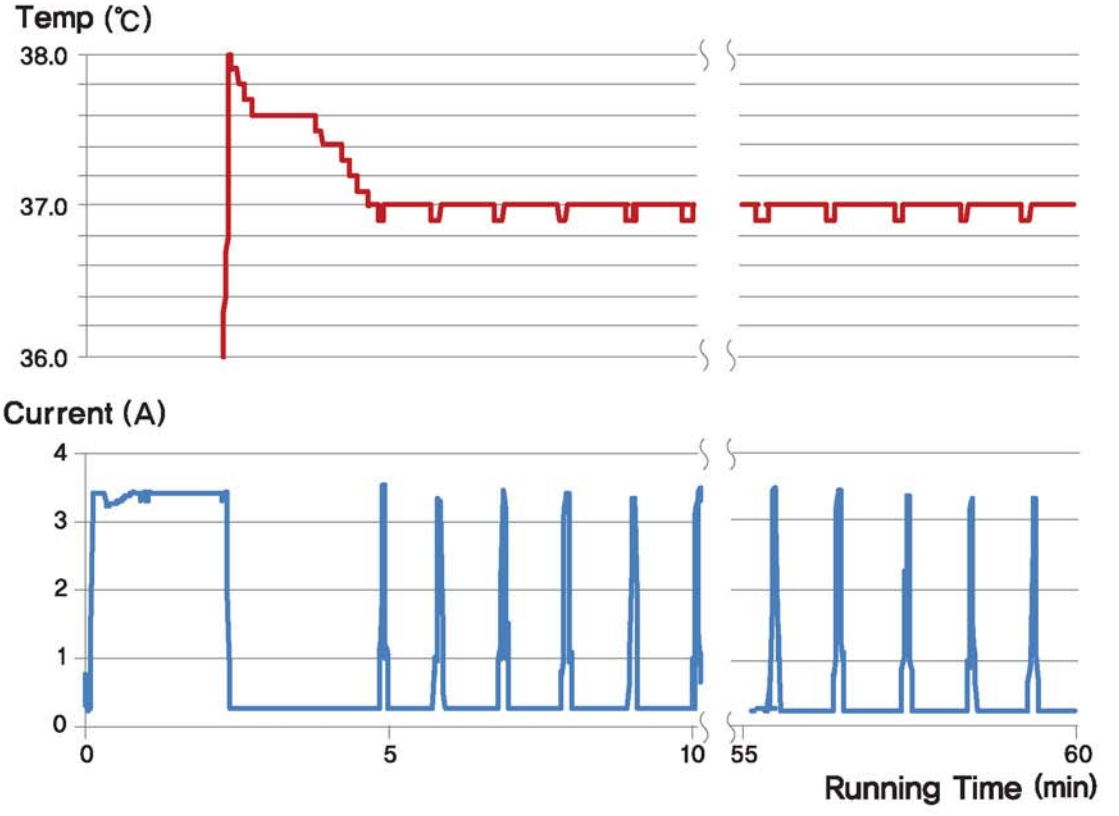
Tips & Trouble Shooting

- Water should be filled before operation.
- Microwave is not emitted if the actual temperature(shown on the REDS) of the water in the Jar is higher than setting temperature.
  ⇨ When the temperature is dropped, then set it again.
- Turn the power off when REDS is not in use.
- Reaction water bath should be empty when REDS is not in use.

◆ Specification

	HST REDS	Other Commercial Product
Microwave Power Range	50 W ~ 950 W	1 W ~ 300 W
Temperature Control Module	Yes	Yes
Versatilities of the Vessels	One Batch can run 35 ea of 1.5 mL Tubes and 48 ea of 0.5 mL Tubes	One Batch can run 14 ea of 1.5 mL Tubes or 20 ea of 0.5 mL Tubes
	96-well plate Available	96-well plate Unavailable
	1 L bottle Available	1 L bottle Unavailable
Temperature Control	Very accurate (± 0.5 ℃)	Not accurate (± 2~5 ℃)
Cooling System	Yes (included)	Yes (optional)
Cooling Unit	Included	Purchased separately

- It takes 3 to 5 minutes for the system to reach the setting temperature of general enzymatic digestions.
- Actual temperature of the system is maintained within ± 0.5 ℃ variation from the setting temperature.



- Once reached to the Set temperature of 37 ℃, it was confirmed that a very regular and even microwave emission at around 2 minutes interval is being supplied by measuring the current at every second.
(Power setting was 600 W, initial Jar temperature was 22.3 ℃.)

◆ Application

1. Digestion of BSA

Material

BSA (66 mg/mL in DW), 45 mM DTT, 100 mM Iodoacetamide, Trypsin (100 ng/㎕ in 50 mM acetic acid), 50 mM NH_4HCO_3, Trifluoroacetic acid(TFA)

Protocol

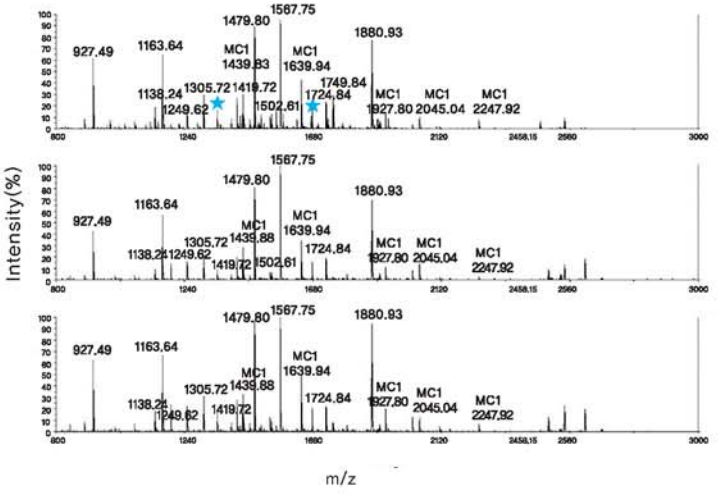
1. Reduction
   ① Preparation of 84 ㎕ BSA(1 nmol) in 50 mM NH_4HCO_3.
   ② Add 5 ㎕ 100 mM DTT and incubate for 30 min at 60 ℃.
2. Alkylation
   ③ Add 5 ㎕ 500 mM Iodoacetamide and incubate for 15 min at RT.
   * Be careful that Iodoacetamide is *light-sensitive!*
3. Enzyme Reaction (Trypsin, Glu-C, Lys-C)
   ④ Add enzyme (100 ng/㎕) 13 ㎕ (enzyme : protein ratio of 1:50) and incubate at 37 ℃ in a water bath for overnight or use the REDS.
4. Ending of the Reaction
   ⑤ Add 5 ㎕ 10% TFA, and then dry the aliquots speedily at reduced pressure.

Analysis of Trypsin digested BSA by MALDI-TOF

In enzymatic reaction, HST REDS has completed the reaction within 10 mins which is faster than water bath. The sequence coverage of HST REDS is better than water bath. The missed cleavage(MC) was confirmed as the same level at both cases. Other enzyme reactions such as Glu-C and Lys-C were also completed within 10 mins by using REDS.

	HST REDS	Conventional (Water Bath)
Protein	BSA	
Enzyme	Trypsin / Glu-C / Lys-C (1:50)	
Reduction and Alkylation	45 mM DTT/ 15 mins at 50 ℃ 100 mM Iodoaetamide/ 15 mins at RT	
Digestion Condition	37 ℃, 10 mins, 400 W	37 ℃, Overnight

Part 1. Trypsin



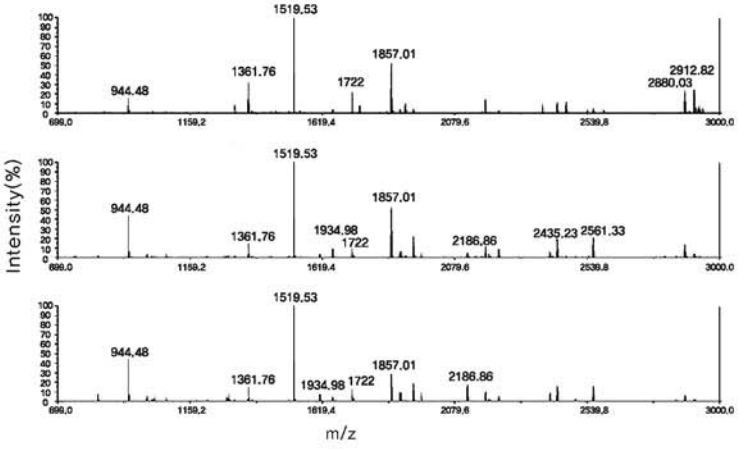
1) 16 h, 37 ℃, Water Bath
Sequence coverage 84%

2) 10 mins, 37 ℃, 400 W, REDS
Sequence coverage 87%

3) 30 mins, 37 ℃, 400 W, REDS
Sequence coverage 89%

★Unknown peak

Part 2. Glu-C

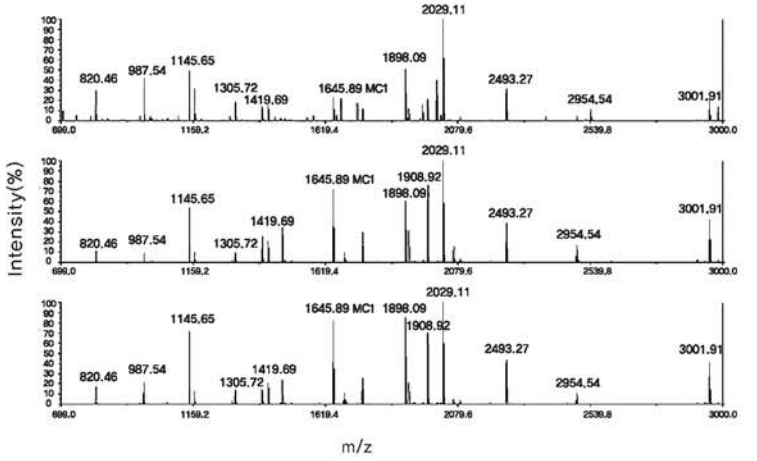


1) 16 h, 37 ℃, Water Bath
Sequence coverage 63%

2) 10 mins, 37 ℃, 400 W, REDS
Sequence coverage 72%

3) 30 mins, 37 ℃, 400 W, REDS
Sequence coverage 78%

Part 3. Lys-C



1) 16 h, 37 ℃, Water Bath
Sequence coverage 68%

2) 10 mins, 37 ℃, 400 W, REDS
Sequence coverage 73%

3) 30 mins, 37 ℃, 400 W, REDS
Sequence coverage 73%

2. Deglycosylation of glycoprotein with PNGase F - RNase B

Material

RNase B (2 mg/mL in DW), 10 mM DTT, 200 mM NH_4HCO_3, PNGase F (NEB)

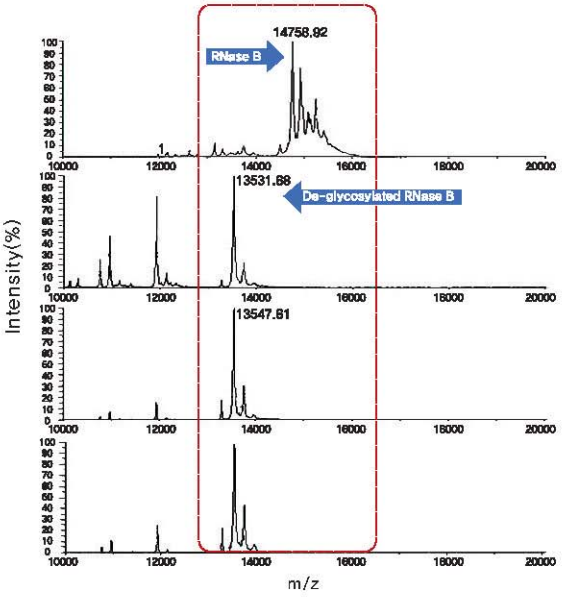
Protocol

1. Reduction
   ① Mix the 50 ㎕ sample and 50 ㎕ buffer (200 mM NH_4HCO_3+10 mM DTT) and vortex for 20 seconds.
   ② Boil the sample for 3 minutes by Alternating between 95 ℃ (15 sec) and 25 ℃ (15 sec)
2. Enzyme Reaction
   ③ Add PNGase F (30 unit) and incubate the reaction at 37 ℃ water bath or use REDS.

RNase B was completely deglycosylated within 10 mins by using HST REDS.

■ RNase B

	HST REDS	Conventional (Water Bath)
Protein	RNase B	
Enzyme	PNGase F (30 unit)	
Digestion Condition	37 ℃, 10 mins, 400 W	37 ℃, Overnight



1) RNase B

2) 16 h, 37 ℃, Water Bath

3) 10 mins, 37 ℃, 400 W, REDS

4) 30 mins, 37 ℃, 400 W, REDS

Mass spectra of RNase B before and after the PNGase F enzymatic digestion. The 100 mg of intact RNase B was digested with PNGase F. Digested samples were mixed with same volume of matrix solution (20 mg/mL sinapinic acid in 30% ACN/0.1% TFA) and loaded onto the MALDI target plate. Intact proteins, glycosylated and deglycosylated, were analyzed using MALDI-TOF MS with linear positive mode.

3. Deglycosylation of glycoprotein with PNGase F - IgG

Material

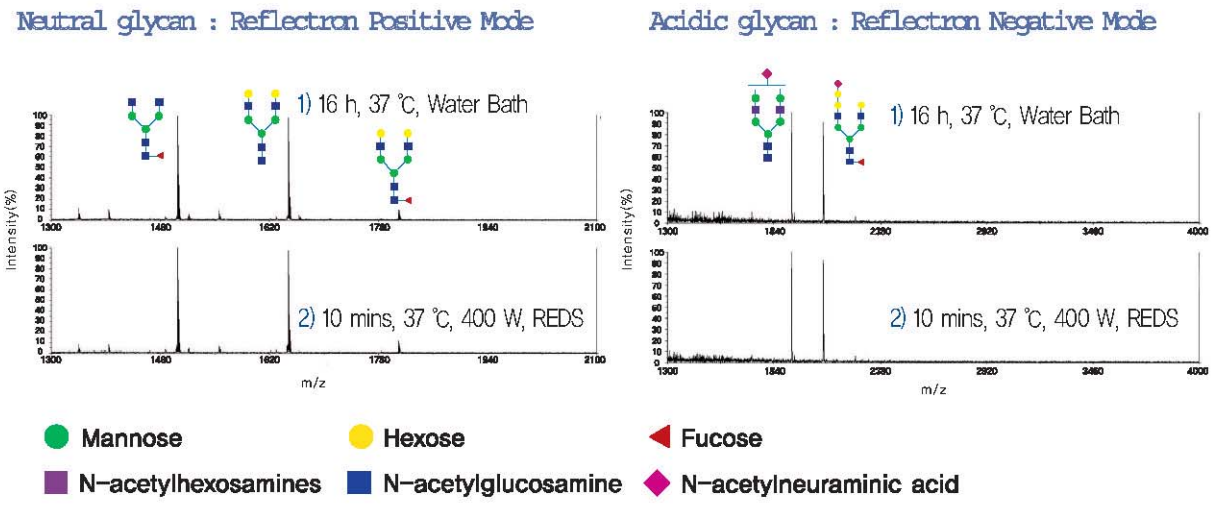
IgG (2 mg/mL in DW), 10 mM DTT, 200 mM NH_4HCO_3, PNGase F (NEB), EtOH, Acetonitrile, Carbon Graphatized Column

Protocol

1. Reduction
   ① Mix the 50 ㎕ sample and 50 ㎕ buffer (200 mM NH_4HCO_3+10 mM DTT) and vortex 20s.
   ② Boil the sample for 3 minutes by Alternating between 95 ℃ (15 sec) and 25 ℃ (15 sec)
2. Enzyme Reaction
   ③ Add PNGase F (500 unit) and incubate it at 37 ℃ water bath or use the REDS.
3. Ethanol Precipitation
   ④ Add 400 ㎕ chilled ethanol.
   ⑤ Freeze to −80 ℃.
   ⑥ Centrifuge for 30 mins at 14000 rpm, 4 ℃.
   ⑦ Transfer the supernatant to a new tube and then dry the aliquots at reduced pressure with high speed.
4. Solid Phase Extraction
   ⑧ Add DW to dissolve the sample and then load it in SPE column. (carbon graphatized)
   ⑨ Elute the sample by (20% / 80%) and (40% / 60%) mixed solvents of Acetonitrile and the water by volume respectively and then speedvac aliquots.
5. Analysis of Sample
   ⑩ Neutral glycan is analyzed with Reflectron Positive Mode while Acidic glycan is analyzed with Negative Mode by applying matrix (DHB was used).

■ IgG

	HST REDS	Conventional (Water Bath)
Protein	IgG	
Enzyme	PNGase F (500 unit)	
Digestion Condition	37 ℃, 10 mins, 400 W	37 ℃, Overnight
Purification	Carbon Graphitized Column SPE	
	Elution Condition : 20% ACN for neutral glycan 40% ACN for acidic glycan	



Mass spectra of the N-linked glycans from IgG released by PNGase F digestion and purified by using Soild Phase Extraction with Carbon Graphite column. MALDI-TOF MS system has been used to characterize the released glycans.

4. Deglycosylation of glycoprotein with PNGase F - Serum

Material

Human serum, 2 mg/mL in DW 10 mM DTT, 200 mM NH_4HCO_3, PNGase F (NEB), EtOH, Acetonitrile, Carbon Graphatized Column

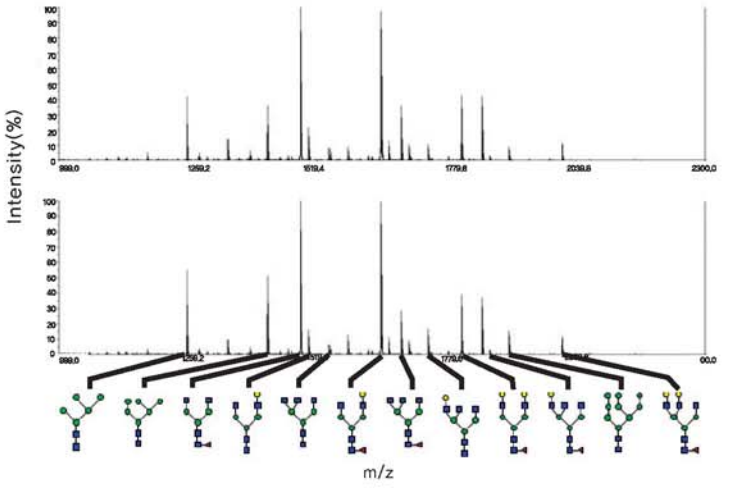
Protocol

1. Reduction
   - ① Mix the 50 ㎕ serum ample and 50 ㎕ buffer (200 mM NH_4HCO_3+10 mM DTT) and vortex 20 seconds.
   - ② Boil the sample for 3 minutes by Alternating between 95 ℃ (15 sec) and 25 ℃ (15 sec)
2. Enzyme Reaction
   - ③ Add PNGase F (500 unit) and incubate it at 37 ℃ water bath or use REDS.
3. Ethanol Precipitation
   - ④ Add 400 ㎕ chilled ethanol.
   - ⑤ Freeze to −80 ℃.
   - ⑥ Centrifuge for 30 mins at 14000 rpm, 4 ℃.
   - ⑦ Transfer the supernatant to a new tube and then speedvac aliquots.
4. Solid phase extraction
   - ⑧ Add DW to dissolve the sample and load into the SPE column (carbon graphatized).
   - ⑨ Elute the sample by 20 % Acetonitrile/water and by 40%, separately and then speedvac the aliquots.
5. Analysis of sample
   - ⑩ Neutral glycan is analyzed with Reflectron Positive Mode while acidic glycan is analyzed with Negative Mode by using matrix (,which is DHB).

Human serum glycans

	HST REDS	Conventional (Water Bath)
Protein	Serum	
Enzyme	PNGase F (500 unit)	
Digestion Condition	37 ℃, 10 mins, 400 W	37 ℃, Overnight
Purification	Carbon Graphitized Column (SPE)	
	Elution Condition : 20% ACN for neutral glycan 40% ACN for acidic glycan	

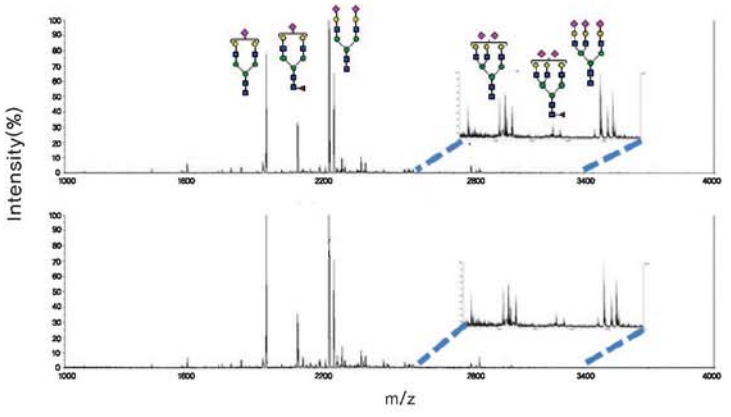
Neutral glycan : Reflectron Positive Mode



1) 16 h, 37 ℃, Water Bath

2) 10 min, 37 ℃, 400 W, REDS

Acidic glycan : Reflectron Negative Mode



1) 16 h, 37 ℃, Water Bath

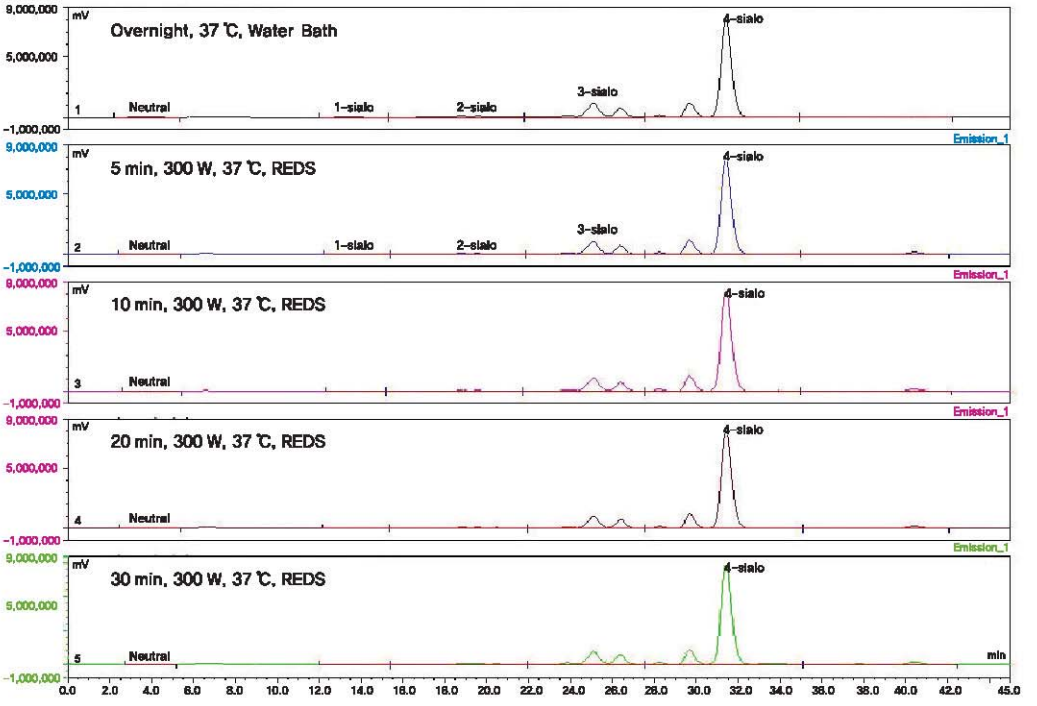
2) 10 mins, 37 ℃, 400 W, REDS

Mass spectra of the N-linked glycans from Human serum glycan released by PNGase F digestion and purified by using Soild Phase Extraction with Carbon Graphite Column. MALDI-TOF MS system has been used to characterize the released glycans.

◆ Appendix

1. Quantitative Analysis of Released Glycans

LC Chromatogram of released neutral and acidic glycans from Protein X



Peak area in chromatogram

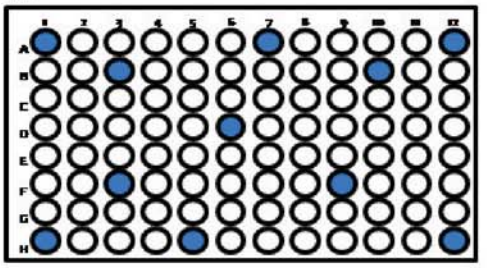
Protein / Prep method	Neutral	Mono	Di	Tri	Tetra	Penta	Z value	SA
Overnight	0.12	0.19	2.79	18.21	75.67	3.03	378.23	18.91
300 W, 5 mins	0.2	0.16	2.55	17.72	76.24	3.14	379.08	18.95
300 W, 10 mins	0.13	0.16	2.5	17.53	76.56	3.12	379.59	18.98
300 W, 20 mins	0.15	0.15	2.44	17.15	77.01	3.09	379.97	19.00
300 W, 30 mins	0.15	0.15	2.36	17.09	77.26	2.99	380.13	19.01
Average	0.15	0.16	2.53	17.54	76.55	3.07	379.40	18.97
S.D	0.03	0.02	0.16	0.46	0.63	0.06	0.77	0.04
RSD%	20.55	10.14	6.44	2.61	0.82	2.04	0.20	0.20

From D pharmacy

Comparison of enzymatic reaction using PNGase F in HST REDS and in a water bath, separately. In enzymatic reaction, both HST REDS for 5 mins and water bath for 16 h brought the same result as above.

2. Homogenous and reproducible data regardless of the location

A)

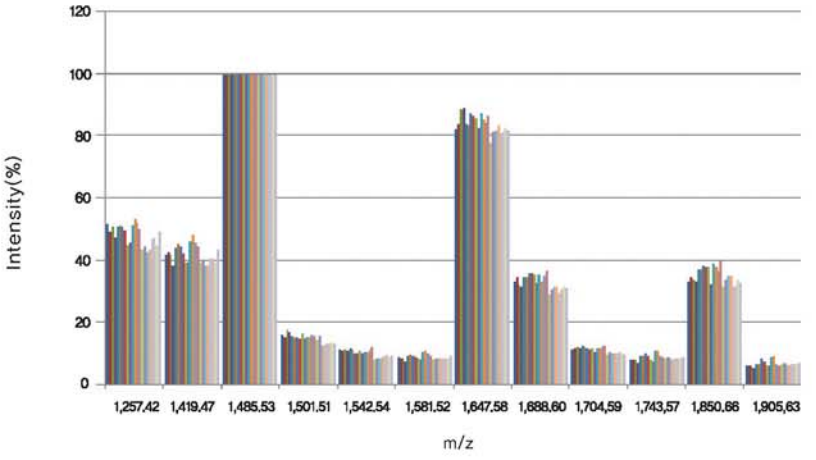


Relative intensity of glycan peaks

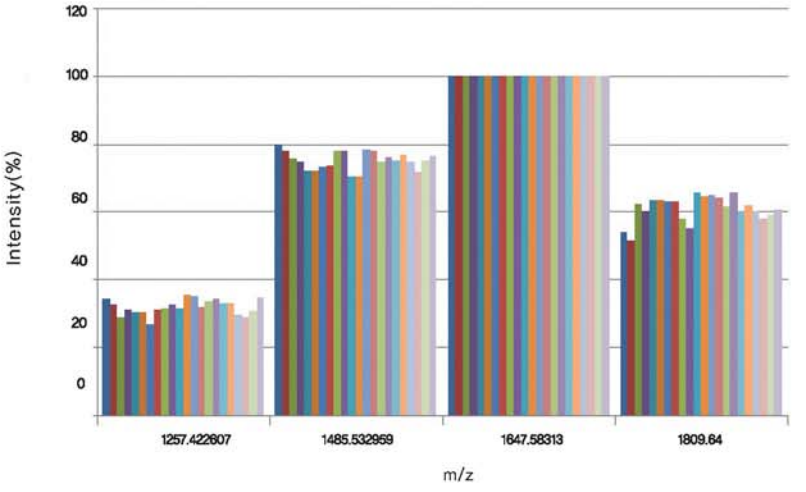
Peak list	AVE	STDEV	CV%
1257.423	48.404	3.243	6.701
1419.473	42.085	2.897	6.885
1485.533	100.000	0.000	0.000
1501.513	15.145	1.437	9.486
1542.539	10.377	1.242	11.971
1581.517	9.015	0.833	9.238
1647.583	84.066	2.770	3.295
1688.602	33.177	2.245	6.766
1704.595	11.395	0.991	8.695
1743.569	8.888	0.973	10.951
1850.664	35.365	2.581	7.298
1905.628	7.032	0.945	13.440

AVE-Average
STDEV-Standard deviation
CV%-coefficient of variation
(Standard deviation / Average *100)

B) 10% fraction



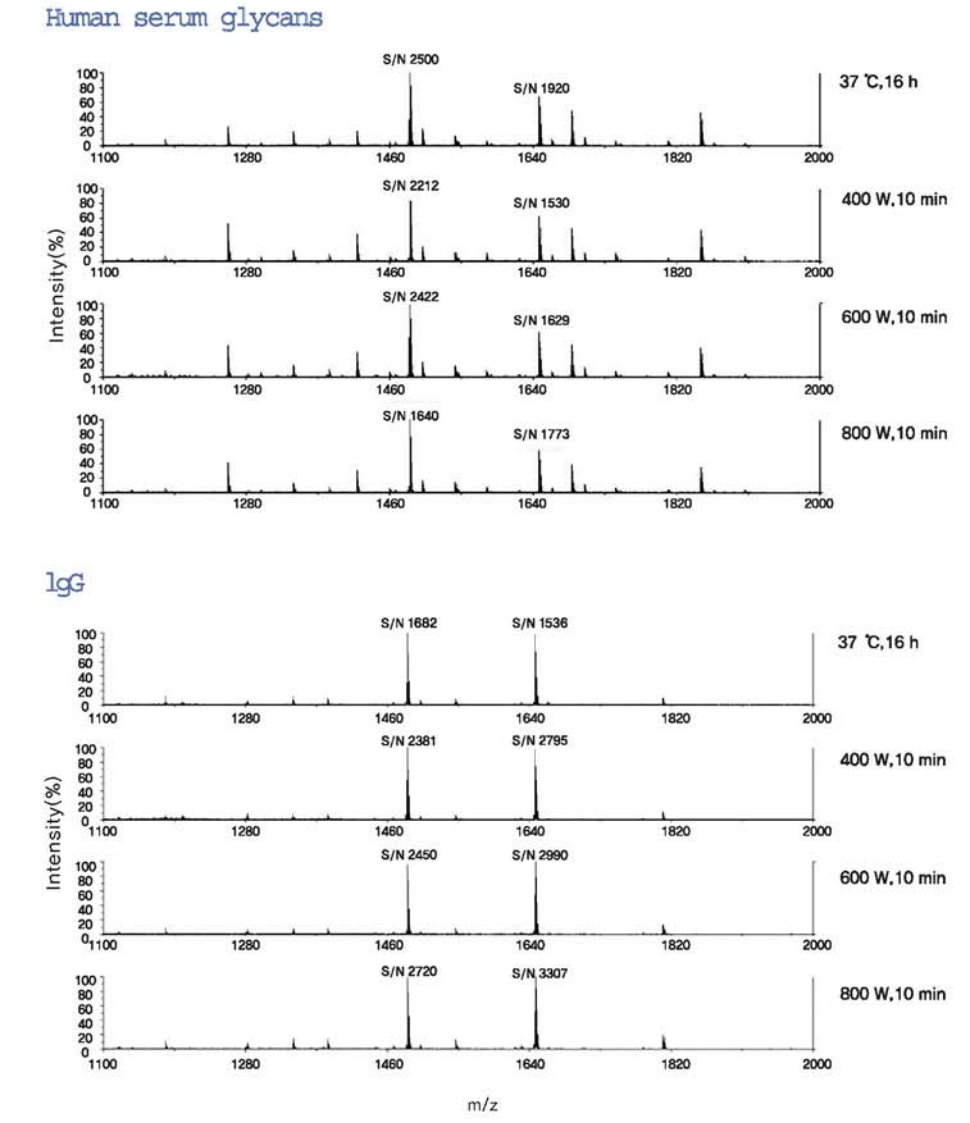
C) 20% fraction



A) The indicated well positions (blue) were tested a in 96 well-plate. B) and C) present normalized intensity of significant peaks, from 10% ACN fraction and from 20% ACN fraction, respectively. Eleven individual samples were analyzed and the reproducible data were obtained regardless of the well positions at the 96 well-plate. Tables present the relative intensity of dominant peaks in each fractions. CV (coefficient of variations) was based on normalized intensity.

3. Homogenous and reproducible data regardless of Microwave power

	HST REDS
Protein	Human Serum Glycan, IgG
Enzyme	PNGase F (500 unit)
Incubation Temperature	37 ℃
Condition	400 W, 600 W, 800 W (10 mins)



Influence of sample and enzyme by Microwave Power (Watt : The figures shows the same peak pattern and the same signal to noise ratio regardless of Microwave Power Setting.