Utilizing Uniform and Reproducible bioZen™ MagBeads for the Immunocapture of Trastuzumab from Rat Plasma

Matthew Brusius
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Overview

In this application, Streptavidin coated bioZen MagBeads are utilized for the fast and accurate immunocapture of trastuzumab from rat plasma. Large molecules have traditionally been analyzed via ligand binding assay, which relative to LC-MS/MS provides narrow LDR, non-linear calibration curves, and time-consuming method development. However, by combining the efficient immunocapture and sample extraction with a highly sensitive and selective MRM, this method demonstrates that it is capable of being multiplexed for higher throughput and lowering background complexity to provide a functionalized assay that yields greater selectivity relative to traditional LBA. Because this method is highly reproducible with low variation between samples, it is ideal for implementation in laboratories.

Materials and Methods

Trastuzumab was purchased from Myoderm (Part No.: 50242-0134-68). Rat plasma was purchased from Bioreclamation-IVT®. Trypsin, TPCK-treated was provided by AB SCIEX® (Part No.: 4445250). Goat Anti-Human IgG-BIOT was purchased from Southern Biotech (Part No.: 2040-08). SiLuMab (Part No.: MSQC3-100UG), Calcium chloride dihydrate (Part No.: C5080), and Trifluoroacetic acid (Part No.: TX1276) were purchased from Sigma-Aldrich®. Strata® Low-Bind 2 mL 96-Well Collection Plates **(Part No.: AH1-7036)** were used for all magnetic bead processing steps. All other reagents were purchased through VWR International LLC.

Sample Preparation

MagBead Activation

1. Aliquot 25 µL of bioZen MagBeads Streptavidin slurry for each sample, 20 mg/mL **(Part No.: KS0-9532)** and wash with 500 µL of PBS Buffer. Discard excess liquid using a magnetic stand (3x).
2. Reconstitute MagBeads to original volume from step 1 using PBS.
3. Add 10 µL of biotinylated goat anti-human IgG (0.5 mg/mL) to the MagBeads for each sample and incubate at room temperature for 1 hour with a shaking speed of 1200 RPM using a deep well plate thermoshaker.
4. Discard excess liquid using a magnetic stand.
5. Wash with 500 µL of PBS Buffer. Discard excess liquid using a magnetic stand (3x).
6. Reconstitute MagBeads to original volume from step 1 using PBS.

Immunocapture

1. Add 50 µL of plasma samples to the wells of a low bind 96-well collection plate. Vortex the MagBeads to thoroughly mix and add 25 µL to each well containing sample.
2. Cover the plate using polyester plate film and vortex to mix before incubating for 1 hour at 1200 RPM on a thermoshaker.
3. Discard excess liquid using a magnetic stand.

Sample Preparation *(con't)*

Washing and Elution

1. Add 200 µL PBS buffer, cover with film, and vortex to mix. Centrifuge at 400 RPM for 2 minutes and discard using a magnetic stand.
2. Add 200 µL of Ammonium bicarbonate and mix using a vortex or pipette. Centrifuge at 400 RPM for 2 minutes and discard using a magnetic stand.
3. Add 100 µL 0.1% TFA in Water and vortex to mix. If applicable, ensure the pH is lower than 3, cover with polyester sealing tape, and shake the plate or tube at 1200 RPM for 10 minutes using a deep well thermoshaker.
4. Centrifuge at 400 RPM for 2 minutes. Place on a magnetic stand for 5 minutes and collect supernatant.

DO NOT DISCARD THE LIQUID.

Protease Digestion of Immuno-Enriched Trastuzumab

1. Transfer eluent (supernatant) into a new low bind 96-well collection plate and discard beads.
2. Add 50 µL of digestion buffer* to each well that contains eluent.
3. Vortex and check pH (should be higher than 7), proceed to cover plate and shake at 300 RPM for 10 minutes at 95 °C using a deep well thermoshaker.
5. Set temperature of thermoshaker to 50 °C and allow plate to cool to < 50 °C.
6. Add 4 µL of 0.25 µg/µL Trypsin in 0.1% Formic acid to each well.
7. Cover, incubate, and shake plate at 300 RPM for 1 hour at 50 °C using a deep well thermoshaker.
8. Centrifuge plate for 2 minutes at 400 RPM.
9. Place plate on 96-well magnetic stand for 10 minutes.
10. Transfer 140 µL of supernatant to an injection plate.
11. Cover and inject 20 µL of each sample for LC-MS/MS.

*digestion buffer = 1mM Calcium chloride in 500 mM Ammonium bicarbonate

LC Conditions

Column: Kinetex® 2.6 µm C18
Dimension: 50 x 3.0 mm
Part No.: 00B-4462-Y0
SecurityGuard™ ULTRA: AJ0-8775
Mobile Phase: **A:** 0.1% Formic acid in Water
B: 0.1% Formic acid in Acetonitrile

Gradient:

Time (min)	% B
0	5
0.7	5
0.8	10
3.5	25
5	40

Flow Rate: 0.7 mL/min
Injection Volume: 20 µL
Detector: SCIEX QTRAP® 6500+

Diverter Valve Switching Program

Time (min)	Position	Location
0	A	Waste
1.5	B	Mass Spec
3.5	A	Waste

MS/MS Detection Source/Gas Parameters

IS: 5500
CUR: 40 psi
TEM: 650°C
GS1: 65 psi
GS2: 65 psi
CAD: High

Compound and Internal Standard MRM Parameters

Trastuzumab	Q1 (m/z)	Q3 (m/z)	DP(v)	EP (v)	CE (v)	CXP (v)	Dwell Time (ms)
FTISADTSK, 2+y7	485.2	721.3	90	10	20	15	40
FTISADTSK, 2+y6	485.2	608.2	90	10	20	15	40
FTISADTSK, 2+b2	485.2	249	90	10	21	15	40
DTLMISR, 2+y5	418.2	619.3	70	10	22	15	40
DTLMISR, 2+y4	418.2	506.2	70	10	23	15	40

SiLuMab (IS)	Q1 (m/z)	Q3 (m/z)	DP(v)	EP (v)	CE (v)	CXP (v)	Dwell Time (ms)
DTLMIS[R], Heavy 1	423.2	629.4	62	10	24	18	40
DTLMIS[R], Heavy 2	423.2	516.3	62	10	24	18	40
[R]: [13C6, 15N4]- Arginine							

Figure 1. Representative Chromatogram of Signature Peptide FTISADTSK, 2+y7 at 50 ng/mL

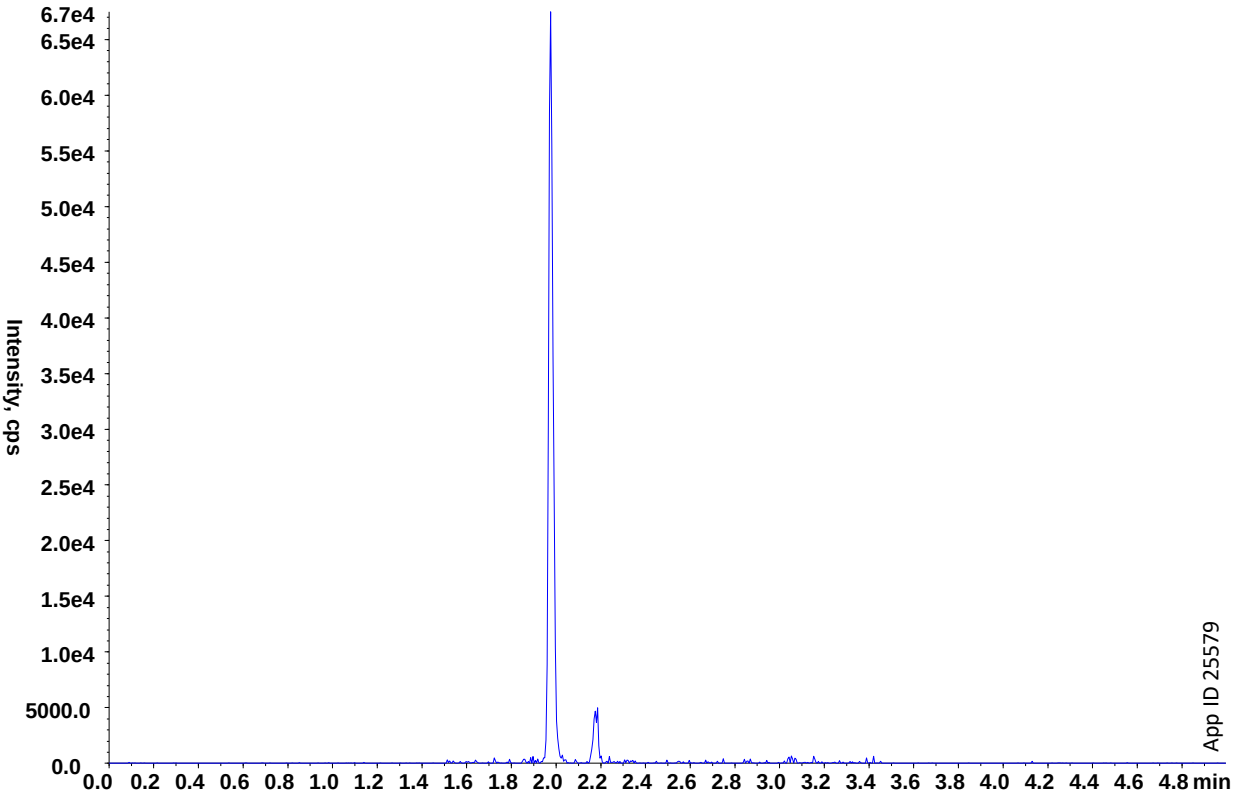
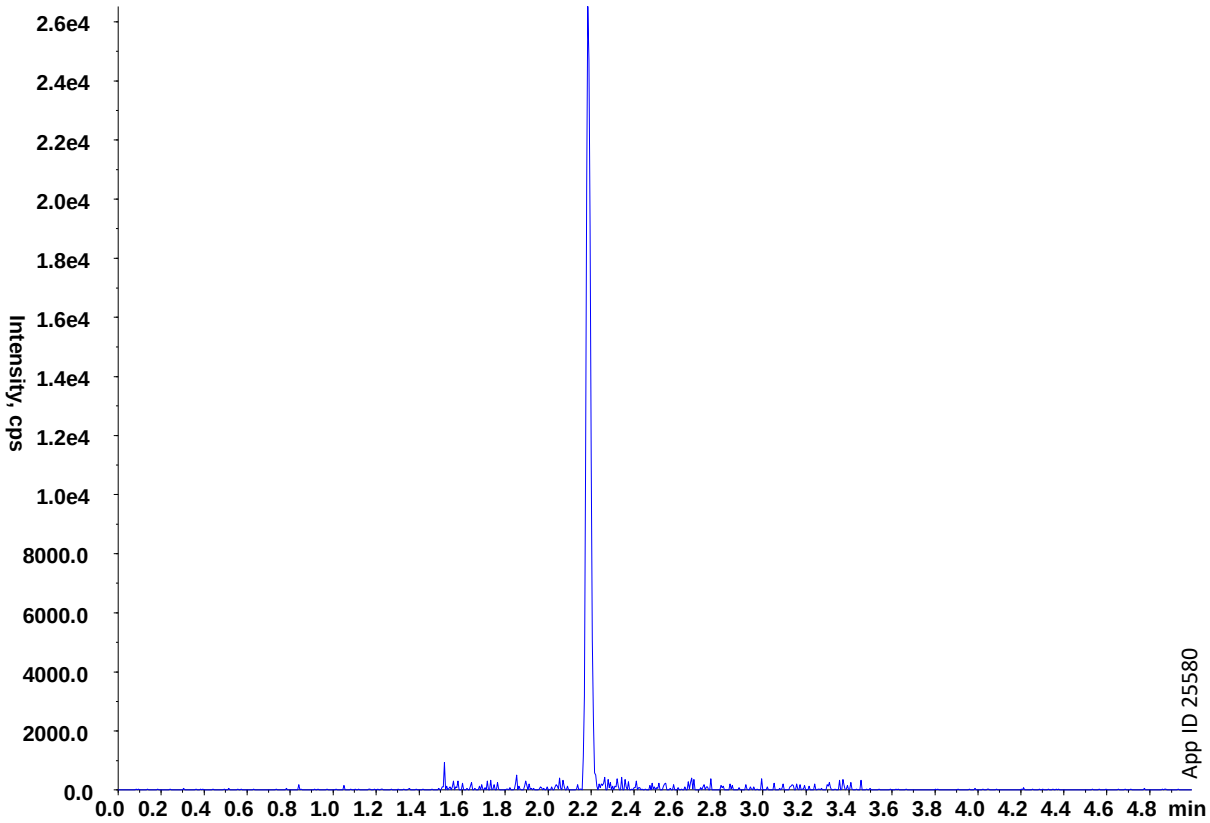


Figure 2. Chromatogram of SiLuMab Signature Peptide DTLMIS[R], Heavy 1 at 50 ng/mL



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Australia
t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
info@phenomenex.com

China
t: +86 400-606-8099
cninfo@phenomenex.com

Denmark
t: +45 4824 8048
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
anfrage@phenomenex.com

India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
nordicinfo@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: +65 800-852-3944
sginfo@phenomenex.com

Spain
t: +34 91-413-8613
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan
t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
info@phenomenex.com

All other countries
Corporate Office USA
t: +1 (310) 212-0555
info@phenomenex.com

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