

# BioBA High Capacity Enrichment Sample Preparation Kit: Biologics Bioanalysis Made Easy

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## Common Immunocapture Sample Extraction Strategies for Biologic Bioanalysis

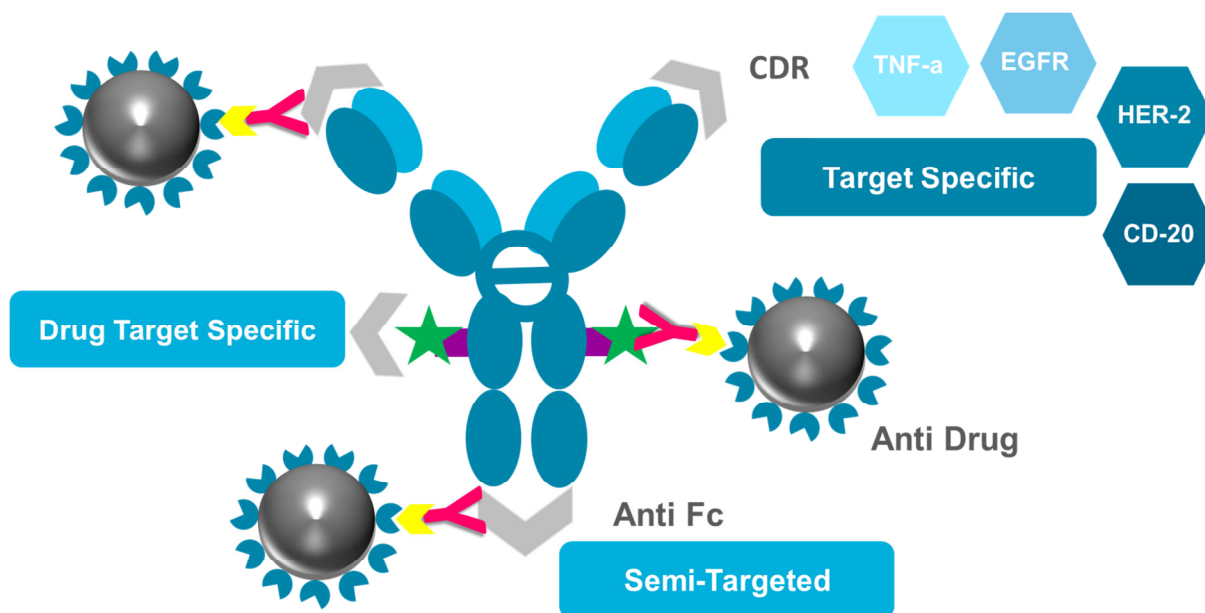
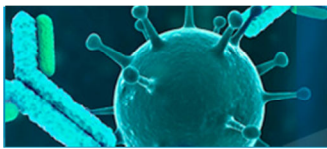


Figure 1: Monoclonal antibody structure showing multiple sample extraction strategies, a semi targeted approach using anti Fc antibody capture, a targeted capture at CDR region using receptor specific antibodies or either anti-drug specific antibody for better specificity

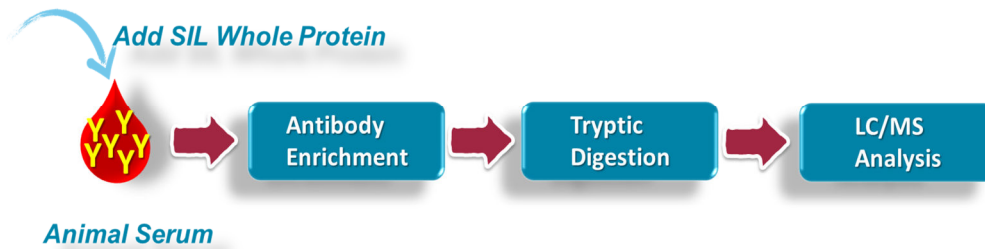
## BioBA High Capacity Enrichment Sample Preparation Kit Selection Guide

	NON-CLINICAL KIT	CORE KIT
<i>Applications</i>	<i>Preclinical Bioanalysis</i>	<i>Both Preclinical/Clinical Bioanalysis</i>
Matrix	Mouse, Rat, Dog, Monkey	Any Matrix
Magnetic Beads	Streptavidin Coated High Capacity Beads	Streptavidin Coated High Capacity Beads
Capture Antibody	Biotinylated Goat Anti Human IgG <i>(included in the kit as separate vial)</i>	Target Specific <i>(not included in the kit)</i>
Binding Site	Fc	CDR
Choice of ISTD	SILu™Mab <i>(Order from Sigma website)</i>	Target specific analogs or heavy labeled analyte
Choice of MRM	Fc region	Fab or CDR region
Specificity		
Selectivity	Semi-Targeted	Highly Targeted
Part Number	5039742	5041071

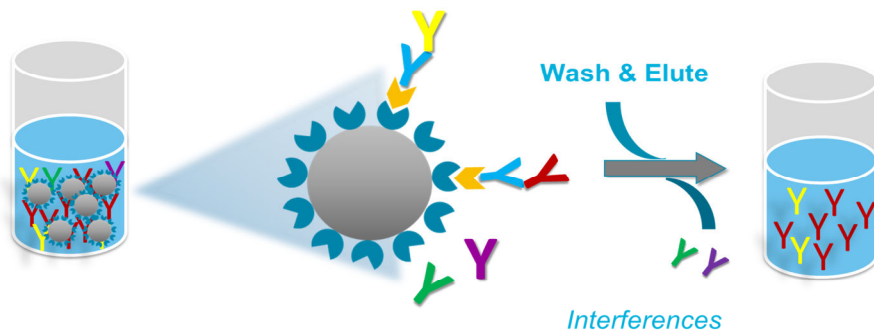


## Easy 3 Step Sample Extraction Process for Sensitive & Selective LC/MS Based Assay

STEP 1:



STEP 2:



STEP 3:

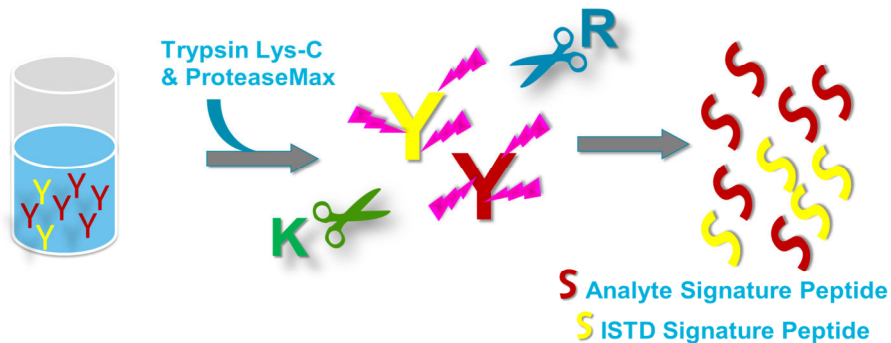


Figure 2: BioBA Sample extraction workflow showing three easy steps to extract monoclonal antibody based therapeutics from complex biological samples such as plasma or serum. Step 1 is to add whole protein stable labeled internal standard to demonstrate QC throughout the sample extraction process, step 2 is capture both internal standard and monoclonal antibody drug using high capacity magnetic beads coated with streptavidin biotin conjugated anti Fc antibody and finally step 3 is digest the monoclonal antibody using Trypsin Lys C enzyme and ProteaseMax surfactant to generate signature peptide for LC/MS quantitation

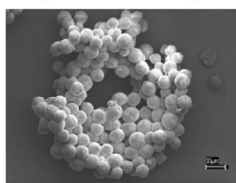
## Universal Internal Standard-SILu™ Mab

- Whole MAb IS reduces error and variability associated with enrichment and enzymatic digestion
- SILuMab is a commercially available stable labeled IgG<sub>1</sub> monoclonal antibody expressed in a CHO cell line
- SILuMab provides utility for the quantification of monoclonal antibodies and Fc-fusion therapeutics
- Surrogate peptides from SILuMab are available for all IgG isotypes in common animal models
- MRM transitions for heavy labeled signature peptides have been developed and can be downloaded from the product website

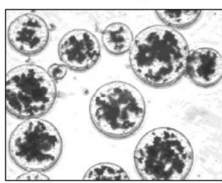
## New High Capacity Streptavidin Coated Magnetic Beads

- Macro porous structure offers high surface area for efficient streptavidin coating
- Low non-specific binding enables higher specificity allowing efficient capture of biotin labeled antibody
- Uniform size and fast magnetic response for easy handling during wash and elution phase

### Magnetic macroporous cellulose particles



SEM Image



Light Microscopy

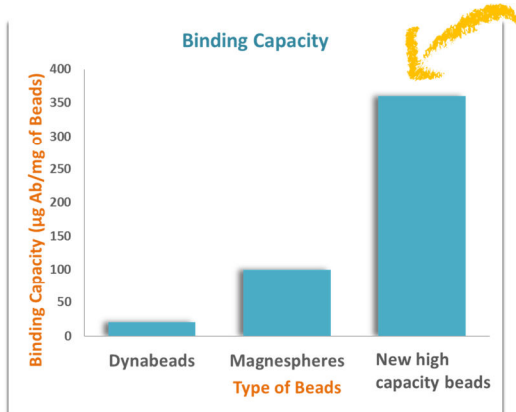
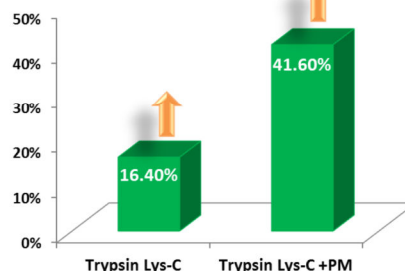


Figure 3: High capacity magnetic beads showing macro porous structure and bar graph showing high capacity approximately 300 µg/mg beads compared to other magnetic beads that are currently available for immunocapture sample extraction

## Trypsin Lys-C and ProteaseMax

- Reduce No. of missed cleavages for better coverage of signature peptide selection during LC/MS method development process
- Protease Max, a MS friendly surfactant enhances digestion efficiency specially of tightly folded and proteolytically resistant biologic drugs & resistant to harsher denaturing conditions
- Wider applicability- all isotypes of IgG (IgG1, IgG2)

% Peptide Coverage Vs. Trypsin Gold Model IgG1



Trypsin Lys-C+ProteaseMax Vs. Trypsin Lys-C (Model IgG1)

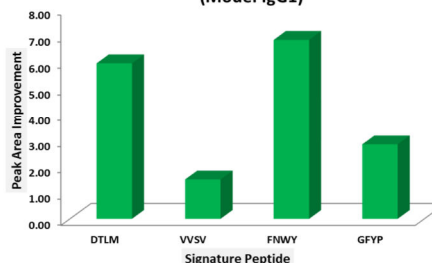


Figure 4: Bar graph showing peptide sequence coverage of a model IgG1. Up to 41% increase in peptide coverage was observed digestion compared to Trypsin Lys C vs Trypsin Lys C with ProteaseMax. Second graph showing an average 2- 7 times increase in peak area of selected signature peptides using MRM based assay

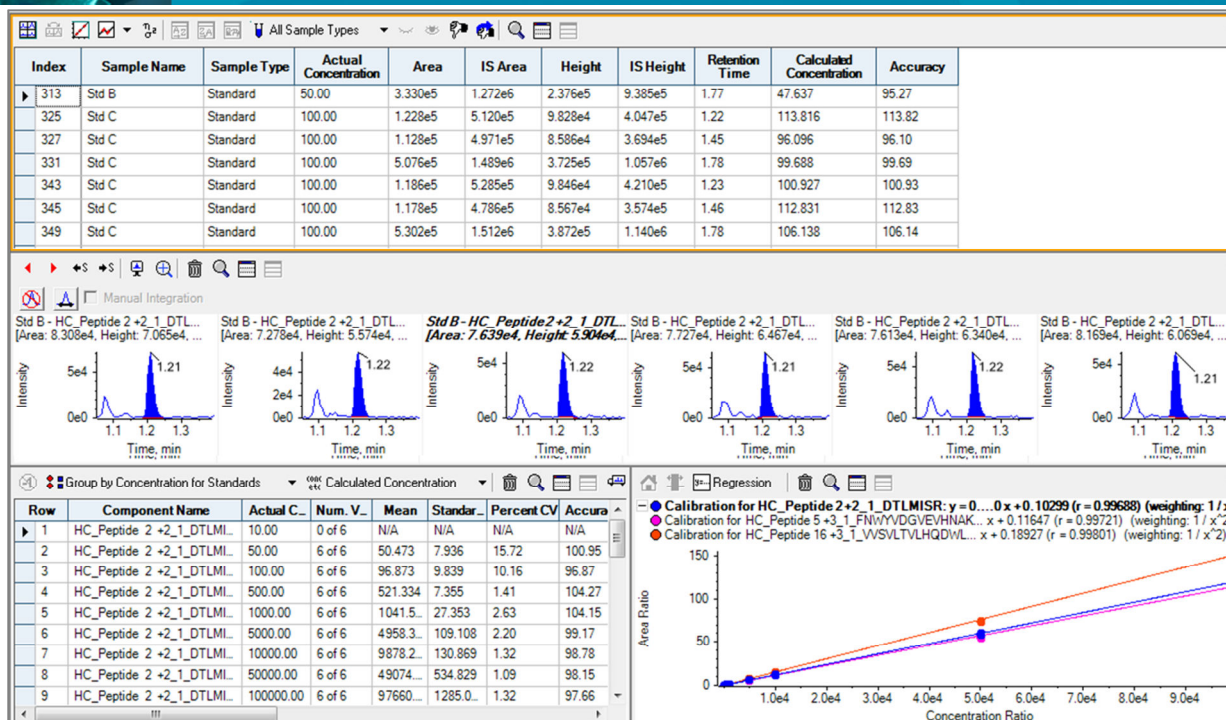
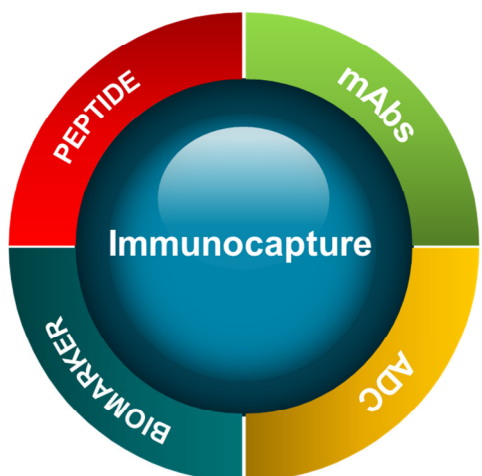
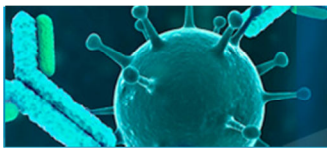


Figure 5. MultiQuant™ Software display of Erbitux signature peptide quantitation results. A screen shot is shown of Erbitux signature peptide quantitation data displayed in MultiQuant™ Software. Panels showing calibration curve concentrations (LOQ-50 ng/mL-ULOQ 100,000 ng/mL), GLP level quantitation statistic % CV < 15.7 & % accuracy 97-104, calibration curve overlays, and chromatographic traces are displayed on one screen for a comprehensive view of signature peptide results. The far left column provides a menu listing every fragment ion for each signature peptide that allows for easier navigation between peptide

## Hyphenating Immunocapture with LC/MS Based Assay



Application	Complementary to LBA
Peptides/ Proteins	Highly sensitive and selective Anti-Insulin antibody based LC/MS Assay
mAbs	Anti Fc or CDR Specific functional assay for free circulating mAb in serum
ADC	Drug target specific In vivo DAR profiling across time course in plasma
Biomarkers	Low abundant protein biomarker bioanalysis in biological matrices



## Biologics Bioanalysis Made Easy

- Ready to use reagents & protocol makes it easy to use
- Universal approach requires minimal method development time
- Highly reproducible performance outfits GLP bioanalysis
- Efficient and generic workflow for biologics bioanalysis

**Easy to Use**

**Universal**

**Reproducible**

**Efficient**

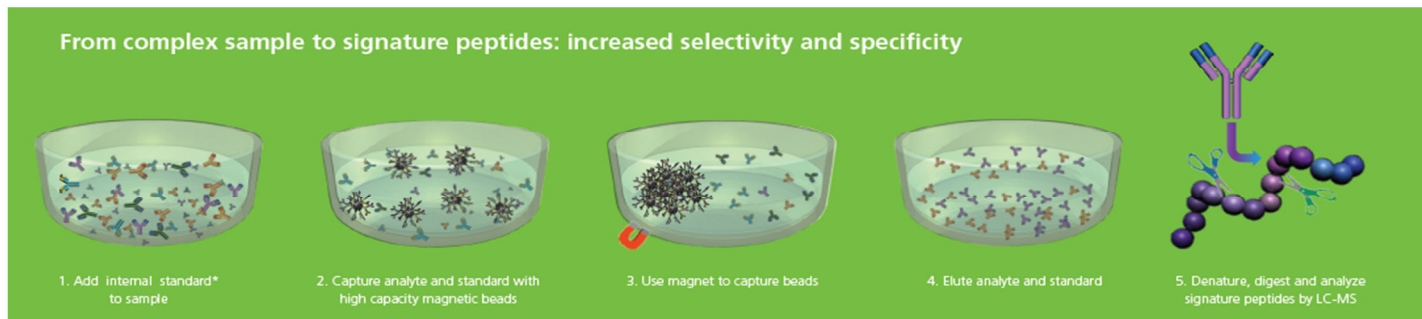
## Ready to use kit for 100 samples

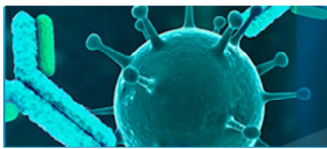


### BioBA Kit Components-

- High Capacity Streptavidin coated Magnetic Beads
- Digestion Enzyme
- Bind, Wash, Elution Buffers & Reagents
- *User Protocol*

### From complex sample to signature peptides: increased selectivity and specificity





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## Drug Discovery and Development



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