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

<table>
<thead>
<tr>
<th>Applications</th>
<th>NON-CLINICAL KIT</th>
<th>CORE KIT</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Preclinical Bioanalysis</td>
<td>Both Preclinical/Clinical Bioanalysis</td>
</tr>
<tr>
<td>Matrix</td>
<td>Mouse, Rat, Dog, Monkey</td>
<td>Any Matrix</td>
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<tr>
<td>Magnetic Beads</td>
<td>Streptavidin Coated High Capacity Beads</td>
<td>Streptavidin Coated High Capacity Beads</td>
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<tr>
<td>Capture Antibody</td>
<td>Biotinylated Goat Anti Human IgG</td>
<td>Target Specific</td>
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<tr>
<td></td>
<td>(included in the kit as separate vial)</td>
<td>(not included in the kit)</td>
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<tr>
<td>Binding Site</td>
<td>Fc</td>
<td>CDR</td>
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<tr>
<td>Choice of ISTD</td>
<td>SiLu™Mab (Order from Sigma website)</td>
<td>Target specific analogs or heavy labeled</td>
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<tr>
<td>Choice of MRM</td>
<td>Fc region</td>
<td>analyte</td>
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<tr>
<td>Specificity</td>
<td>Semi Targeted</td>
<td>Fab or CDR region</td>
</tr>
<tr>
<td>Selectivity</td>
<td>Part Number</td>
<td>Highly Targeted</td>
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<td>Part Number</td>
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<td>5041071</td>
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p 1
Easy 3 Step Sample Extraction Process for Sensitive & Selective LC/MS Based Assay

STEP 1:

Add SIL Whole Protein

Animal Serum

STEP 2:

Wash & Elute

Interferences

STEP 3:

Trypsin Lys-C & ProteaseMax

Analyte Signature Peptide
ISTD Signature Peptide

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, 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

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)

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

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

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

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

1. Add internal standard* to sample
2. Capture analyte and standard with high capacity magnetic beads
3. Use magnet to capture beads
4. Elute analyte and standard
5. Denature, digest and analyze signature peptides by LC-MS
Please use this space for your notes.