RepliGen
BIO PROCESSING

Dilute & Go™
Protein A Detection ELISA

Sometimes Different IS Better

Repligen's Protein A ELISA Kits for detection of leached Protein A are chosen by biopharmaceutical companies for their easy-to-use procedure, quick run time, sensitivity, and accurate results. Our kits are used in process development, process monitoring, and in final product testing to detect and quantify natural, recombinant Protein A, and MabSelect SuRe™ ligand contamination in samples eluted off of a Protein A chromatography column.

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The combination of a highly specific capture system and an optimized sample diluent yields a highly sensitive assay able to accurately detect Protein A in the presence of antibodies without the time and resources required for in-house assay development and optimization.

Quality organizations recognize that for the most accurate results the best practice is to incorporate a standard “spiking” protein that is most like the contaminant ligand. For this reason Repligen has developed two distinct Protein A detection assays, one for the detection of natural and recombinant Protein A and another for the detection of the MabSelect SuRe™ ligand.

Sample preparation is simplified by a Dilute and Go strategy, characterized as being compatible with most common Protein A elution buffers.

Typical Applications:

Monoclonal Antibodies: Process Development, Manufacturing Product Quality and Contaminant Monitoring

Our Protein A detection ELISA kits are designed for in-process monitoring and final product testing in a wide variety of antibody preparations. It has been developed for those customers who require a highly sensitive assay to measure small amounts of contaminating Protein A in antibody products. The combination of a unique and highly specific capture system and an optimized sample diluent yields a highly sensitive assay able to accurately detect Protein A in the presence of antibodies.

Testing for Protein A contamination occurs in several different phases of development and commercial manufacturing:

- Process development for leaching characteristics of the resin under specific conditions
- Manufacturing, typically from eluted samples taken throughout several points in the purification process
- Final product release to document process contaminate levels and lot-to-lot consistency

Limits for leached Protein A in process development and monitoring samples are typically set internally but limits for finished product release are generally set in collaboration with regulatory agencies.

Although the USP does not recommend a specific commercial kit for Protein A ELISA detection, Repligen’s leached Protein A assays are chosen by global biopharmaceutical companies for their easy to use procedure, quick run time and accurate results. Our commercially available kit reduces assay development time and resources required for in-house assay development. In addition, Repligen is the provider of two separate kits, one for the detection of native and recombinant Protein A and another for the detection of the MabSelect SuRe™ ligand. For the most accurate results, standards and spiking proteins should match the contaminant ligand.

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Contract Manufacturing Operations

A feature of contract manufacturing operations is that many of the manufacturing processes are therapeutic specific and typically transient in nature as they move through clinical manufacturing to commercial scale. While it may make sense to develop in house ELISA assays for large scale manufacturing operations that are run on a routine basis, for small scale and more transient manufacturing processes a generic kit approach is almost always the faster, cheaper and more reliable approach.

Repligen's Dilute & Go leached protein A ELISA kits are ideal for CMO's conducting contract process development and clinical manufacturing. Dilute & Go kits provide a fast, reliable and cost effective off the shelf option for validation, process contamination monitoring and product release testing of leached Protein A.

Feature
Simplified "Dilute & Go™" sample preparation.

Benefit
- Fast & Easy "out of the box"
- No buffer dialysis
- Reduced preparation time for faster results
- Eliminates expensive dialysis membranes
- Better sample integrity

Feature
Uniquely optimized sample diluent

Benefit
- Precise
- Reliable accurate and precise measurement of contaminating Protein A in Mab product pool

Feature
Contaminant matched standards

Benefit
- Specific
- Separate ELISA for the detection of Protein A or MabSelect SuRe™ ligand.

Feature
Optimized capture and detection antibodies

Benefit
- Sensitive
- Low limit of Quantitation ≤ 1 ppm
- Reduced non-specific signal

Feature
Well characterized assay performance

Benefit
- Qualified, reliable product quality testing
- Repligen protein A ELISA kits used in release testing of several commercial and clinical stage monoclonal antibodies and Fc Fusion protein therapeutics

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Now Even Greater Convenience - Dilute & Go™ Protocols

NEW - Our newly introduced Dilute & Go protocol further simplifies and quickens our assay procedure by eliminating dialysis or buffer exchange for most sample matrices. Reducing sample preparation time and minimizing protein loss!

A sample taken from a purified Protein A eluate or final product pool is diluted directly to an antibody concentration of 0.5 mg/ml using a phosphate buffered saline with 0.1% tween20 diluent. This step is a substitute for dialysis into PBS followed by dilution to 0.5 mg/ml.

Dilute & Go Method Criteria:

1. **The following common sample buffers were verified to be compatible:**
   - 100 mM Glycine
   - 100 mM Acetate
   - 100 mM Citrate
   - All buffers were prepared at a pH of 3.0 and neutralized to pH7.6 with Tris-base. Each contained 5 mg/ml of human polyclonal IgG.

2. **Antibody Concentration ≥ 5 mg/ml**
   Buffering system ≤ 100 mM, if a higher buffering capacity is used it is possible to perform a direct dilution so long as the final buffer concentration is less than 2.5 mM.

3. **Following the new Dilute & Go step, the assay is then performed according to standard kit instructions.**

   To prepare these samples with the Dilute & Go method simply:
   - Dilute purified in-process and/or finished product antibody samples directly into phosphate buffered saline (PBS) with 0.1% tween20 to reach a sample concentration of 0.5 mg/mL
   - Proceed to the Preparation of Sample Dilutions section of the user guide

Note: No dialysis is required when the Dilute & Go step is performed.

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Dilute & Go™
Protein A Detection ELISA

Sensitive sub-Part per Million Level Detection

Has enabled Repligen ELISA products to be reliably used for product quality and process contaminant release testing of several commercial and clinical stage monoclonal antibodies and Fc Fusion protein therapeutics. Although there is no specific regulatory guidance the FDA requires that manufacturers demonstrate that there are very low levels of contaminants in the final therapeutic formulation. The high reproducibility demonstrated by the Repligen ELISA kits for Protein A recovery across a range of model buffer matrices shown in tables 1 & 2 coupled with the demonstration of sub part per million (ppm) quantitation levels highlights the capability and suitability of the Repligen kits for this critical release testing.

Dilute & Go ELISA Assay Performance

As well as being fast and easy Repligen's Dilute & Go™ ELISA is importantly, sensitive, specific and highly reproducible. These key performance characterisitics are critical to the effectiveness and applicability of the assay to therapeutic product and process quality monitoring and release.

The following sections and tables demonstrate the critical performance capability of the Protein A assays when using the Dilute & Go method. In all cases the sample buffer/hIgG matrix was tested in triplicate at 6 different Protein A input levels in three independent assays. The recovery, precision (inter and intra assay) and lower limit of quantitation was determined for both the rPA and MabSelect SuRe™ kit using the modified assay procedure. Results show the Protein A assays continue to provide excellent recovery and sensitivity results when the Dilute & Go method is used.

table 1

Assay Recovery & Sensitivity of the rPA Ligand

Buffer	Range Tested (ng/ml)	Mean Recovery(%)	High (%)	Low (%)	LLoQ (ng/ml)	LLoQ (ppm)
Citrate	1.6 - 0.05	98	103	91	0.038	0.3
Glycine	1.6 - 0.05	101	102	96	0.067	0.54
Acetate	1.6 - 0.05	97	100	90	0.033	0.26

table 2

Assay Recovery & Sensitivity of the MabSelect SuRe™ Ligand

Buffer	Range Tested (ng/ml)	Mean Recovery(%)	High (%)	Low (%)	LLoQ (ng/ml)	LLoQ (ppm)
Citrate	1.6 - 0.05	101	105	95	0.058	0.46
Glycine	1.6 - 0.05	99	102	94	0.051	0.41
Acetate	1.6 - 0.05	101	103	94	0.046	0.37

LLoQ is calculated at 10 times the standard deviation of the background.
NOTE: LLoQ Lower Limit of Quantitation

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Dilute & Go™
Protein A Detection ELISA

High Confidence of Detection of Contaminant in Antibody Pool

Recovery of the Protein A contaminant at low detection limits throughout the detection range of the assay is critical for dependable accurate reporting of results. Repligen ELISA assays kits show robust and highly reproducible recovery of Protein A. The measured recoveries are consistent across the entire dynamic range of the assay when a protein A spike is added to immunoglobulin containing buffers (Figures 1a &1b). These results show the assays specificity for Protein A despite the presence of other proteins.

figure 1a

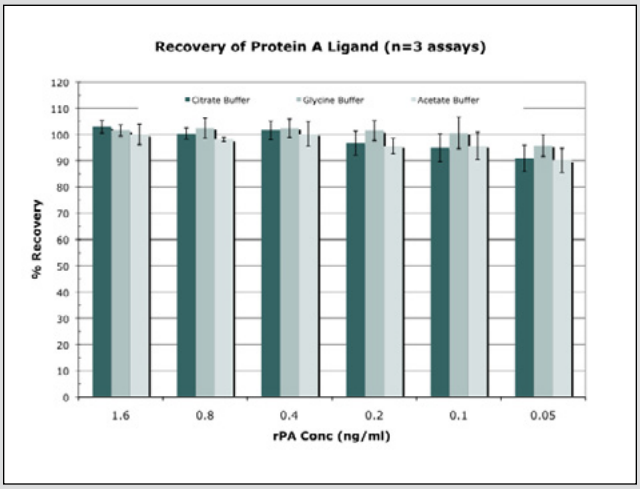
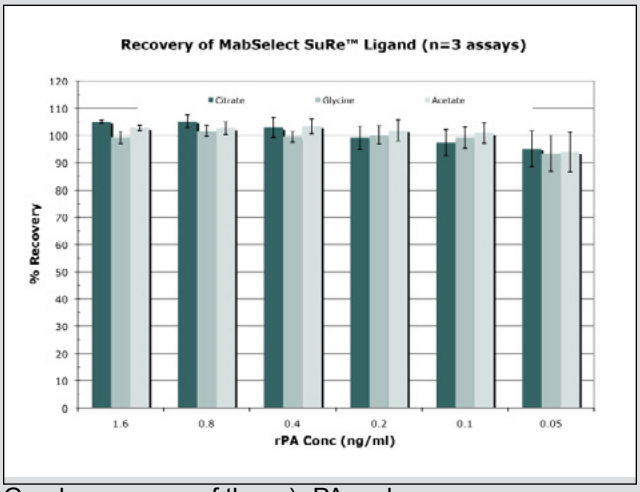


figure 1b



Graphs recovery of the a.) rPA and b.) MabSelect SuRe™ ligand when spiked at different levels into hIgG containing model buffer matrices

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Dilute & Go™
Protein A Detection ELISA

Validatable Leached Protein A ELISA Platform

For an analytical test to be used for product quality release it must be robust, reliable and validated. This means that the test must give reproducible results from replicates within and between test data. In the case of a 96 well plate based assay like the Repligen Protein A ELISA this means inter and intra plate consistency. This reproducibility is clearly demonstrated in tables 3 & 4 which show a high degree of precision (%CV) throughout the dynamic range of the assay within replicate samples and assays across a range of model buffers.

Note: Due to protein sample variation, Repligen recommends that end users fully characterize assay performance with their proprietary antibody products.

table 3

Assay Precision (%CV) when detecting the rPA Ligand

rPA (ng/ml)	Citrate Intra	Citrate Inter	Glycine Intra	Glycine Inter	Acetate Intra	Acetate Inter
1.6	2.1	2.3	1.7	2.1	3.5	3.9
0.8	1.8	2.1	1.8	3.7	2.1	0.8
0.4	3.3	3.5	2.8	3.5	2.3	4.8
0.2	2.1	4.7	0.9	3.7	2.4	3.1
0.1	4.9	5.4	5.6	6.0	2.6	5.3
0.05	3.7	5.3	4.7	4.5	3.0	5.1

table 4

Assay Precision (%CV) when detecting the MabSelect SuRe™ Ligand

SuRe™ (ng/ml)	Citrate Intra	Citrate Inter	Glycine Intra	Glycine Inter	Acetate Intra	Acetate Inter
1.6	2.7	0.6	1.6	2.2	2.7	0.9
0.8	4.4	2.2	1.8	2.0	3.5	2.2
0.4	1.7	3.6	2.4	2.0	1.9	2.6
0.2	3.9	4.2	2.0	3.3	2.1	3.9
0.1	2.5	5.0	2.4	3.9	2.0	3.7
0.05	6.3	6.9	3.2	6.9	3.0	7.6

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ELISA KIT – Detection of Leached Native and Recombinant Protein A

Repligen's Protein A ELISA kits have been used by technology leaders in our industry for years to ensure lot-to-lot consistency in their monoclonal antibody and Ig fusion products. Appropriate for processes run on Protein A columns that contain all natural and recombinant Protein A resins except for the MabSelect SuRe™ ligand.

Easy to Use:

- **NEW Dilute & Go protocol**
  - Faster sample preparation
  - Minimize loss of protein
  - Optional adoption, no re-validation required
- **Ready to go "out of the box", no plate coating required**
- **Plug and play for process development, process monitoring, and quality control**
- **No individual reagent qualification**
- **No methods development required**

Product Features Include:

- **Sensitivity of 0.1 ng/mL or 0.8 ppm**
- **Accuracy/Recovery 80-120%**
- **Precision: Inter-assay 10%, Intra-assay 15%**
- **Specificity for all variants of natural and recombinant Protein A**
- **2 hour assay completion time**

Call 781 250-0111 to Order

ELISA Kit For the Detection of Leached Natural and r ProteinA ligands

Catalog Number	Description
9000-1	Protein A ELISA for detection of natural and recombinant Protein A

RepliGen BIO PROCESSING

Dilute & Go™
Protein A Detection ELISA

ELISA KIT – Detection of Leached MabSelect SuRe™ ligand

Repligen's Protein A ELISA kits have been used by technology leaders in our industry for years to ensure lot-to-lot consistency in their monoclonal antibody and Ig fusion products. For downstream processes that utilize the base stable affinity media MabSelect SuRe™, Repligen offers the only kit on the market that includes the SuRe™ ligand standard for the most accurate spiking and recovery results.

Easy to Use:

- **NEW Dilute & Go protocol**
  - Faster sample preparation
  - Minimize loss of protein
  - Optional adoption, no re-validation required
- **Ready to go "out of the box", no plate coating required**
- **Plug and play for process development, process monitoring, and quality control**
- **No individual reagent qualification**
- **No methods development required**

Product Features Include:

- **Sensitivity of 0.1 ng/mL**
- **Accuracy/Recovery ± 80-120%**
- **Precision: Inter-assay 10%, Intra-assay 15%**
- **Specificity for MabSelect SuRe™ ligand**
- **2 hour assay completion time**

Call 781 250-0111 to Order

ELISA Kit For the Detection of Leached MabSelect SuRe™ ligand

Catalog Number	Description
9333-1	Protein A ELISA for detection of MabSelect SuRe™ ligand

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