

Protein ELISA Kits



Repligen's Protein A ELISA Kits deliver the reproducibility, precision, and sensitivity required for the accurate detection and quantification of leached native protein A, recombinant protein A, and MabSelect SuRe™ ligand, NGL-Impact™ A ligand and LONG® R³ IGF-I in downstream processing applications.



ACCURATE
Most accurate matched standard for MabSelect SuRe™ resin



REPRODUCIBLE
Consistent inter- and intra-kit consistency



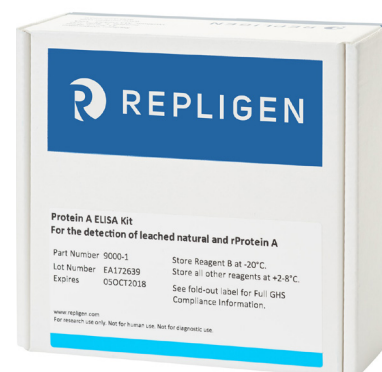
HIGH SENSITIVITY
using biotinylated rabbit and anti-Protein A detection antibody



HIGH SPECIFICITY
Using polyclonal chicken capture antibody

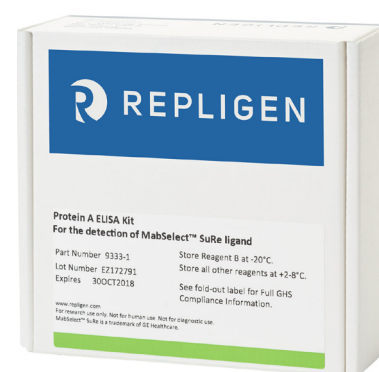


OPTIMIZED
With three proven sample preparation protocols



The Recombinant Protein A ELISA Kit delivers the most reproducible ELISA platform for detection and quantitation of leached recombinant and native protein A. As the world's leading supplier of protein A, a large percentage of the world's commercial supply of antibody relies on a Repligen manufactured protein A ligand for purification.

Repligen is the only protein A manufacturer who provides an ELISA kit to accurately and reproducibly detect leached protein A.



The protein A construct in the MabSelect SuRe™ affinity resins is the least similar to other commercially available protein A ligands. Using traditional protein A ELISA kits the SuRe™ ligand is not accurately quantitated.

For downstream processes which utilize the base stable affinity resin MabSelect SuRe™, Repligen offers the only kit on the market which includes the SuRe™ ligand standard for the most accurate, precise, and sensitive measurements.

Superior Reproducibility with Repligen Protein A ELISA Kits

Protein A ELISA Kits

Process Development
Is my process designed well?

Process Monitoring
Is my process running properly?

Product Quality
Is my product consistent, lot-to-lot, batch-to-batch?

Product Release
Is my drug product safe when it leaves my facility for patient dosing?

Repligen

Features & Benefits

- Superior inter and intra kit consistency for unparalleled reproducibility
- Polyclonal chicken capture antibody for greater protein A specificity
- Biotinylated rabbit anti-protein A detection antibody for greater sensitivity
- Matched standards for the greatest quantitation accuracy
- Three sample prep protocols for superior method optimization

A large percentage of the world's commercial supply of antibody relies on a Repligen manufactured protein A ligand for purification and Repligen's ELISA kit for product release.

Protein A ELISA Kit for MabSelect SuRe™ Ligand



Part Number : 9333-1

For downstream processes that utilize a MabSelect SuRe™ capture step, Repligen offers the only kit on the market that includes the SuRe™ ligand standard for the most accurate quantitation.

- Exact matched Protein A reference standard (SuRe™ ligand)
- Sensitivity of 0.1ng/mL
- Recovery : 80-120%
- Accuracy & Precision : Inter-assay +/-10%, Intra-assay +/-15%

Kit Contents

- Anti-protein A coated 96 well microtiter plate
- Reagent A : sample diluent (5x)
- Reagent B : MabSelect SuRe standard solution
- Reagent C : rabbit anti-protein A : biotin probe
- Reagent D : streptavidin : peroxidase conjugate
- Reagent E : TMB peroxidase substrate
- PBS packs (quantity 2)

Protein A ELISA Kit for NGL-IMPACT™ A Ligand



Part Number : 9222-1

For downstream processes that utilize a Praesto® Jetted A50 capture step, Repligen offers the only kit on the market that includes the NGL-Impact™ A Ligand standard for the most accurate quantitation.

- Exact matched Protein A reference standard (NGL-Impact™ A Ligand)
- Sensitivity of 0.1ng/mL
- Recovery : 80-120%
- Accuracy & Precision : Inter-assay +/-10%, Intra-assay +/-15%

Kit Contents

- Anti-protein A coated 96 well microtiter plate
- Reagent A : sample diluent (5x)
- Reagent B : NGL-Impact™ A standard solution
- Reagent C : rabbit anti-protein A: biotin probe
- Reagent D : streptavidin: peroxidase conjugate
- Reagent E : TMB peroxidase substrate
- PBS packs (quantity 2)

Protein A ELISA Kit for Native and Recombinant Protein A



Part Number : 9000-1

For downstream processes which utilize a Protein A capture step (non-MabSelect SuRe™), Repligen offers the Protein A ELISA Kit suitable for Protein A resins that contain native and recombinant Protein A ligand variants.

- Specificity for all variants of native and recombinant Protein A
- Sensitivity of 0.1ng/mL or 0.8ppm
- Recovery : 80-120%
- Accuracy and precision : Inter-assay +/-10%, Intra-assay +/-15%

Kit Contents

- Anti-protein A coated 96-well microtiter plate
- Reagent A : sample diluent (5x)
- Reagent B : recombinant protein A standard solution
- Reagent C : rabbit anti-protein A : biotin probe
- Reagent D : streptavidin : peroxidase conjugate
- Reagent E : TMB peroxidase substrate
- PBS packs (quantity 2)

ELISA Kit for the Detection of LONG® R³ IGF-I



Part Number : 9547-1

The LONG® R³ IGF-I ELISA Kit is the only commercially available kit for the quantitation of LONG® R³ IGF-I. Fast, accurate, and easy to use with both cell culture and drug substance samples, this sandwich ELISA kit uses a biotinylated secondary antibody and Streptavidin-HRP for detection.

- Limited reactivity to native or recombinant IGF-I
- No reactivity to insulin
- Ready-to-use ELISA plate allows the assay to be run immediately without additional plate coating and blocking steps

Kit Contents

- LONG® R³ IGF-I coated strip plate, 96 wells
- LONG® R³ IGF-I Standard
- Diluent
- Biotinylated detection antibody
- Streptavidin Peroxidase
- TMB Peroxidase Substrate
- LoBind® 1.5mL Eppendorf tubes

Sample Preparation Protocols

Optimized Methods for Accurate Detection and Quantification of Leached Protein A

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. This eliminates the need for tedious in-house assay development and optimization.

Three Simple Sample Preparation Protocols

Repligen has designed three sample prep protocols to maximize ease of use, speed, and limits of detection and quantification. Both the Recombinant Protein and MabSelect SuRe™ kits are compatible with all three protocols.

Dilute & Go™

- Maximize ease of use and assay run time with no buffer exchange step
- Sample can be diluted DIRECTLY into the assay*

Boil & Boost™

- Increase sample input concentration and decrease limit of quantitation
- Combines the low pH dissociation step with boiling (no buffer exchange step**)

Original Protocol

- Suitable for legacy methods
- Requires a buffer exchange step

* Must evaluate specific matrices for feasibility

** Some buffers may require dialysis in the Boil & Boost protocol

Dilute & Go™ Protocol

A sample taken from a purified protein A eluate or final product pool is diluted directly to an antibody concentration of 0.5mg/ml using a phosphate buffered saline with 0.1% tween 20 diluent. This step is a substitute for dialysis into PBS followed by dilution to 0.5mg/mL.

Sample Criteria :

Antibody Concentration : $\geq 5\text{mg/mL}$, buffering system $\leq 100\text{mM}$

If a higher buffering capacity is required, direct dilutions can be performed as long as the final buffer concentration is less than 2.5mM.

After the dilution step, the assay is performed according to standard kit instructions.



Dilute & Go™ Case Study

The following pages demonstrate the critical performance capability of the Dilute & Go method and Repligen's Protein A ELISA kits. In all cases the sample buffer/hlgG matrix was tested in triplicate at 6 different protein A concentration levels in three independent assays. The recovery, precision (inter and intra assay) and lower limit of quantification was determined for both the Recombinant Protein A and MabSelect SuRe™ kits.

The common buffers below were verified to be compatible :

- 100mM Glycine
- 100mM Acetate
- 100mM Citrate

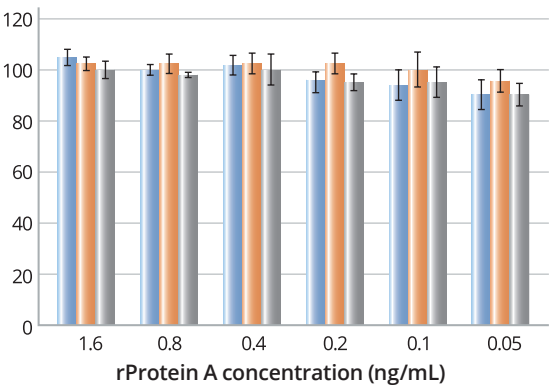
All buffers were prepared at a pH of 3.0 and neutralized to pH 7.6 with Tris-base. Each contained 5mg/mL of human polyclonal IgG.

Protein A Detection Superior Dynamic Range

Detection of leached Protein A at low limits is critical for accurate testing and reliable Protein A ELISA Kits from Repligen deliver consistent recovery performance throughout the detection range of the assay across different process buffers.

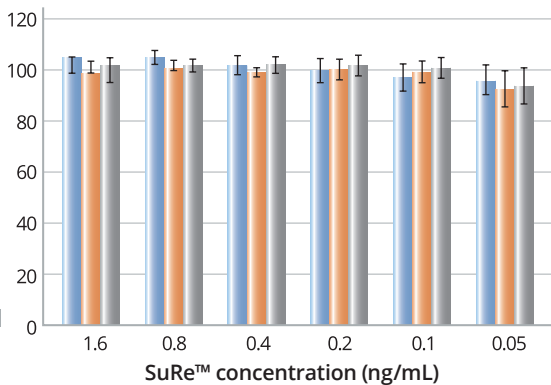
Recovery of rProtein A Ligand

(Recombinant Protein A ELISA Kit, n=3 assays)



Recovery of MabSelect SuRe™ Ligand

(MabSelect SuRe™ ELISA Kit, n=3 assays)



The measured recoveries are consistent across the entire dynamic range of the assay when a protein A spike is added to immunoglobulin containing buffers. These results show the assay's specificity for protein A despite the presence of other proteins.

Sub ppm Level Detection Protein A Quantitation

Although the FDA does not publish a limit on allowable levels of protein A, manufacturers must characterize and quantitate the levels of protein A contaminants in the final therapeutic formulation. Because of their high assay recovery and sensitivity, Protein A ELISA kits from Repligen are used in the product quality and process contaminant release testing of many commercial/clinical stage monoclonal antibodies and Fc Fusion protein therapeutics.

Assay Recovery & Sensitivity : Recombinant Protein A Ligand

Protein A ELISA Kit for Native and rProtein A						
Buffer	Protein A Range Tested (ng/ml)	Mean Recovery (%)	High (%)	Low (%)	LLOQ (ng/mL)	LLOQ (ppm)
Citrate	1.6 -0.05	98	103	91	0.038	0.30
Glycine	1.6 -0.05	101	102	96	0.067	0.54
Acetate	1.6 -0.05	97	100	90	0.033	0.26

Assay Recovery & Sensitivity : MabSelect SuRe™ Ligand

Protein A ELISA Kit for MabSelect SuRe™ Ligand						
Buffer	Protein A 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

Reproducibility Inter- & Intra- Kit Consistency

The Protein A ELISA Kits from Repligen demonstrate critical inter- and intra- kit consistency. Reproducibility is demonstrated below, with the high degree of precision (%CV) throughout the dynamic range of the assay within replicate samples and across three model buffers.

Assay Precision (%CV) :

Detection of the Recombinant Protein A Ligand

(Recombinant Protein A ELISA Kit, n= 3 assays with triplicate wells)

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

Assay Precision (%CV) :

Detection of the MabSelect SuRe™ Ligand

(MabSelect SuRe™ ELISA Kit, n= 3 assays with triplicate wells)

rPA (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

Experience the Difference Reliable, Robust, and Reproducible



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