

# Protein A ELISA

This ELISA kit is designed to detect native and recombinant Protein A from *Staphylococcus aureus* (SpA), in samples such as antibody preparations. The kit will detect the MabSelect SuRe™ ligand from GE Healthcare.

Many immunoglobulins bind Protein A non-specifically via the Fc region. This Protein A ELISA kit utilizes IgG from chicken, also known as IgY, which is one of the few immunoglobulins that does not bind Protein A in the Fc region.



## Features and Benefits:

- Superior accuracy: Boiling procedure gives recoveries close to 100%.
- Broad application range: Dynamic range is at least 3 logs and the kit can be used for samples with or without IgG.
- High sensitivity: Sensitivity is 0.15ng/mL even at IgG concentrations up to 1mg/mL.
- Cost effective and flexible: 96 well plate in a 8 x 12 strip format.

## Typical Applications:

- For manufacturers of Protein A columns: Quality control of leached material.
- For manufacturers of monoclonal antibodies: Quantification of Protein A in the antibody solutions after purification.
- For research and diagnostics: Verify absence of Protein A in samples to avoid false results in immunological assays.

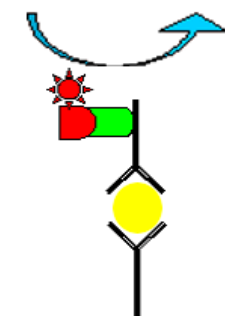
### Ordering Information

Product	Quantity	Product No.
Protein A ELISA	8 x 12 strips	03-96

## Sample Preparation Principle

Samples tested are often of acidic pH. At this low pH, the specific immunological detection of SpA is decreased rendering poor assay performance. This is overcome by neutralizing the samples and standards.

Also, SpA may be non-specifically bound to IgG which falsely lowers the signal. To avoid and normalize the effect of this problem, IgG is added to standards. Standards and samples containing IgG are then treated to denature the IgG, thus maximizing the exposure of SpA epitopes.



### Test Procedure, samples with IgG

1. Normalize samples and standards with respect to IgG.
2. Remove IgG by boiling.
3. Add 100  $\mu$ L of standards and samples, incubate 60 min.
4. Wash strips.
5. Add 100  $\mu$ L of Biotinylated anti-SpA IgY, incubate 60 min.
6. Wash strips
7. Add 100  $\mu$ L of HRP conjugate, incubate 30 minutes.
8. Wash strips
9. Add 100  $\mu$ L of Substrate solution, incubate 10 min.
10. Add 100  $\mu$ L of Stop solution.
11. Read absorbance at 450 nm.

### Summary of Performance

Assay time	3 hours
Sensitivity (LOD)	0.15 ng/mL (ppm)
CV, intra assay	5%
CV, inter assay	15%
Specificity	>75%, depending on the source <sup>1</sup>
Linearity	0.15 – 33 ng/mL (ppm)
Matrix effect	Not seen <sup>2</sup>
Antigen excess (hook effect)	Not seen for samples tested up to 1600 ppm
Recovery	85-115%

<sup>1</sup> Recovery of multiple native and recombinant sources tested.

<sup>2</sup> When assay is normalized by addition of IgG to the standards. Source should be similar to the IgG present in the samples.

## References

- Larsson A. et al., A microELISA useful for determination of SpA-binding monoclonal antibodies. *Hybridoma*, 9, 289-294 (1990).
- Lindmark R. et al., Binding of immunoglobulins to SpA and immunoglobulin levels in mammalian sera. *J. Immunol. Methods*, 62, 1-13 (1983).
- Forsgren, A., Ghetie, V., Lindmark, R., Sjöquist, J., SpA and its exploitation. In: *Staphylococci and Staphylococcal infections 2* (Eds. C.S.F. Easmon and C. Adlam), pp. 429-480, Academic Press Inc., London 1983.