DATA SHEET

Protein A Resin

cat. no.	amount	note
STS-PA	10ml	Protein A Agarose resin, 50% ethanol

Protein A Resin is useful for affinity purification and isolation of IgG. Protein A, a bacterial cell wall protein isolated from Staphylococcus aureus, binds to mammalian IgGs mainly through Fc regions. Native Protein A has 5 IgG binding domains and many others domains of unknown functions. Recombinant Protein A contains mainly five high affinity (Ka=108/M) IgG binding domains with others non essential domains removed to reduce nonspecific binding. Additionally a 3×Cys tag was engineered to the C-terminal of rec-protein A to facilitate its immobilization or conjugation.

SPECIFIC APPLICATIONS:

- immunoglobulin purification
- Chromatin Immunoprecipitation (ChIP)

PROPERTIES OF StoS ProtA Resin:

Ligand	Recombinant Streptococcal protein A produced in E.Coli
# of IgG binding sites per ligand	5
MW of ligand	Approx. 43kDa
PI of ligand	5,17
Degree of substitution	Approx. 2mg ProtG/ml
Static binding capacity	>20mg sheep IgG/ml drained medium
Stability	2-8°C, 6 months
Matrix Spherical	Agarose, 4%
Average particle size	90µm (45-165µm)
Storage solution	1x PBS containing 20% ethanol
Storag conditions	2 - 8°C

*For research only, not for resale

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IMMUNOGLOBULIN PURIFICATION PROCEDURE:

Before use, prepare the following two solutions:
1. Binding Buffer A: Na_2HPO_4 20 mM
   NaCl 0.15 M, adjust pH to 7.0.
2. Elution Buffer B: Citric acid 0.1 M, adjust pH to 3.

This procedure is for a column of 0.5 ml bed volume. The volumes of reagents can be scaled up or down according to the size of the column.
1. Mix the slurry by gently inverting the bottle several times to suspend the resin completely.
2. Use a pipette to transfer appropriate volume of Protein A Resin slurry to a column. Allow the resin to settle and the storage buffer to drain from the column.
3. Add 5 ml of Binding Buffer A to equilibrate the Protein A Resin.
4. Dilute the sample with the same volume or more of Binding Buffer A before applying onto the Protein A column to maintain optimal ionic strength for binding.
5. Wash the column with 10 ml of Binding Buffer A.
6. Elute the antibody with 10 ml of Elution Buffer B. Immediately neutralize the eluted fractions with 1 M Tris-HCl, pH 8.5 to pH 7.4.

Regeneration of the column.
1. Regenerate column by washing the column with 10 ml of Elution Buffer B followed by equilibration of the column with 5 ml of Binding Buffer A. Columns can be regenerated up to 10 times without significant loss of binding capacity.
2. For storage, wash column with 5 ml of PBS containing 0.02% sodium azide. Store column upright at 4°C.

Chromatin Immunoprecipitation:

NOTE: only with polyclonal Abs

Protein A pre-clearing
* IMPORTANT: before S To S Protein A pre-clearing, wash twice protein A with IP buffer*.
* For each sample, incubate 15 µl of S To S Protein G slurry with 1 µg/µl salmon testis DNA and 1 µg/µl BSA (up to 100 µl with IP buffer) in a rotating wheel for 2 hours at 4°C. Centrifuge 4000 rpm 2min, discard supernatant and incubate o.n. with the same amounts of st DNA and BSA.

Chromatin pre-clearing
* First, pre-equilibrate 40 µl S To S Protein A slurry with 40 µl IP buffer + PIC per sample in as many tubes as different chromatins you're going to use. Centrifuge 4000 rpm 2min (you'll get approx. 25-30 µl of S To S Protein G pellet/sample) and resuspend it again with 40 µl IP buffer + PIC per sample. Add chromatin (for each sample pre-clear 100 µl of chromatin) and incubate for 2 hours on a rotating wheel at 4°C.

Chromatin + antibodies incubation
* Spin down the pre-cleared chromatin at 4000 rpm 2 min, collect and transfer supernatant aliquots to pre-siliconated 0.5ml-PCR tubes (as many as different antibodies you want to test), add 2-8 µg of the appropriate antibody to each tube and bring the final volume to 200-300 µl with IP buffer + PIC. Incubate o.n., rotating at 4°C.

Immunoprecipitation
* Spin down pre-cleared, stDNA- and BSA-saturated S To S Protein A, discard supernatant and resuspend it with IP buffer + PIC per sample. Aliquot 110 µl of S To S Protein A in each tube containing chromatin + antibodies.Incubate chromatin + antibodies + S To S Protein G for at least 2 hours, rotating at 4°C.
* Centrifuge samples at 4000 rpm 2 min. Remove supernatant.
* Wash the resin. After the last wash, remove any traces of buffer (if necessary, quickly spin down again) and resuspend the resin with 100 µl 1x TE**pH 8.

*IP buffer: 1M TrisHCl pH 8.0, 0.5M EDTA, 20% SDS, 10% deoxycholic acid, 1M LiCl, 200xPIC
**1X TE: 10 mM. Tris-HCl, 1 mM EDTA, pH 8.0

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