

3. Wash wells 3 times and add 100ul of primary antibody (non-biotinylated) or primary antibody-HRP conjugate diluted in sample/conjugate diluent; incubate at RT for > 1h.

*[Primary antibody binds to Ag on well: sandwich]*

4. HRP-Ab: Wash 5 times and proceed to TMB step.

Primary Ab: Wash 3 times and add 100ul of secondary antibody diluted in sample/conjugate diluent; incubate at RT for >1h. Wash 5 times.

5. Add 100ul TMB and incubate at RT x 15-30min.

*[HRP converts TMB to readable signal proportional to Ag concn]*

6. Add 100ul Stop Solution, read absorbance at 450nm and process data.

**Related Material available from ADI**

Catalog #	Description
PRTG55	Recombinant Protein G Coated Microplates
PRTA55	Recombinant Protein A Coated Microplates
80160	Rabbit Antibody Detection ELISA Kit
40321	Mouse Antibody Detection ELISA Kit (others available)
80050	Coating Buffer (10X), 25 ml
80062	Blocking Buffer (10X), 25 ml
80070	Sample/Conjugate Diluent (10X), 25 ml
80081	Wash Solution Concentrate (100X), 25 ml
80091	TMB Substrate Solution for HRP, Ready-to-Use, 100ml
80100	Stop Solution for HRP (10X), 10 ml
SVDN11-R-1	Purified >98% (S. Avidinii) Streptavidin protein
SV-PETR-100	Streptavidin-PE-TR (Texas red) conjugate
20365	Streptavidin-Peroxidase (HRP) conjugate
20366	Streptavidin-AP (Alk Phosphatase) conjugate
20367	Streptavidin-Fluorescein (FITC) conjugate
20368	Streptavidin-Rhodamine (TRITC) conjugate
20369	Streptavidin-Phycoerythrin (PE) conjugate

## Streptavidin Plates

Cat. No. SVDN55-5P

### Streptavidin Coated 96-well Microplates

*[for capturing biotinylated proteins, peptides, and DNA]*



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## INTRODUCTION

Streptavidin is a non-glycosylated polypeptide chain having a molecular mass of 240 kDa and containing 4 subunits, with each having a binding site for biotin. The streptavidin is isolated by purification from fermentation broth. The neutral isoelectric point of streptavidin and the high affinity of the biotin-streptavidin interaction results in rapid binding kinetics with high signal-to-background ratios. When used in biological assays, streptavidin does not react non-specifically with endogenous lectins.

Streptavidin Coated Microplates, Cat# SVDN55-5P, are coated with highly purified streptavidin. The plates are post-coated (pre-blocked) to further reduce non-specific binding, maintain stable activity, and to provide the convenience of direct use.

Applications for the use of the plates include the following:

- Capture and analysis of antibodies using labeled antigens
- ELISA
- Protein-protein interactions
- Isolation and analysis of fusion proteins or native proteins

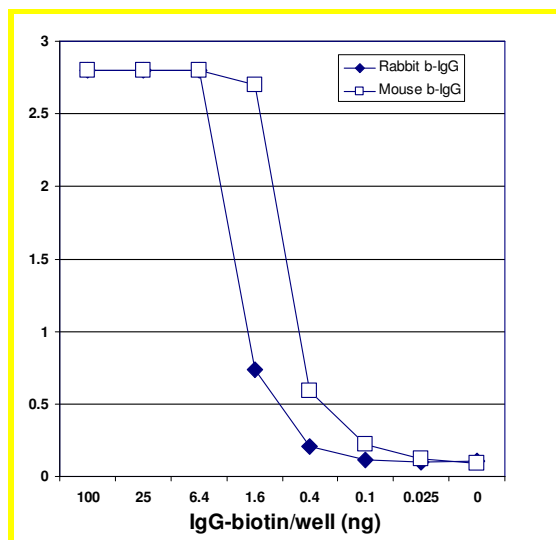


Figure 1. Capture of biotinylated rabbit and mouse IgG

## SPECIFICATIONS

### Components

Five (5) individually pouched 96-well plates, configured in 12 removable 8-well strips.

### Coating

Purified Streptavidin is coated using 100ul/well. Nominal binding capacity is ~5 pmol biotin/well. The strips are post-coated for low non-specific binding and long-term stability.

### Sensitivity

Biotinylated IgG was detected at concentrations significantly above background in an ELISA format using anti-rabbit or mouse IgG-HRP (at 0.1 ug/ml) as detector and TMB as substrate (see Figure 1), as follows:

Rabbit IgG – 0.6 ng/well

Mouse IgG – 0.4 ng/well

### Storage and Stability

The microplates, if unopened, are stable refrigerated until the expiration date printed on the label. If opened, store in closed pouch with desiccant and use within 2-4 weeks.

## APPLICATION

### Strategy for Antigen Quantitation by ELISA

#### Materials Required

- Streptavidin Coated Plate, Cat# SVDN55-5P
- Biotinylated primary antibody to specific antigen of interest
- Cell lysate or soluble preparation containing specific antigen of interest
- Enzyme-labeled (HRP or ALP) primary antibody, or primary antibody + enzyme-labeled secondary antibody
- ELISA diluents and wash, enzyme substrate & stopping solutions

Note: Rabbit (Cat# 80160) and Mouse (Cat# 40321) Antibody Detection Kits are available from Alpha Diagnostic, Intl, and contain the specified secondary HRP conjugate with all required reagents for performing about 10 plates of ELISA assays.

#### Protocol

1. Add 100ul of biotinylated primary antibody, diluted in coating solution, to the Streptavidin coated wells; incubate for >1 hour.  
[Ab-biotin binds to streptavidin on well]
2. Wash wells 3 times (250ul of wash solution) and add 100ul of dilutions of samples, standards, and/or controls, diluted in sample diluent, to appropriate wells; incubate at room temperature for >2 hours, or overnight at 4° C.  
[Ag binds to primary antibody on well]