Streptavidin-HRP Conjugate (ELISA Grade)

PRODUCT ANALYSIS SHEET

Catalog Number:	SNN2004
Lot Number:	See product label
Quantity/Volume:	1 mg
Presentation:	Purified streptavidin-horseradish peroxidase conjugate in phosphate buffered saline with calf serum and 50% glycerol.
Preservation:	0.01% thimerosal (Caution: thimerosal is a poisonous substance. Handle with care and dispose of properly.)
Suggested Working Dilutions:	This conjugate should be diluted in a buffer appropriate for use with ELISA just prior to use. The suggested working dilution is 0.10 to 0.80 micrograms per milliliter with the recommended use of 100 µL per well. The optimal concentration should be determined for each specific application. A general procedure including composition of diluent buffer (Standard Diluent Buffer/Assay Buffer) is attached.
Storage:	Store this preparation at 2-8°C for up to one month. For long term storage, apportion into working aliquots and store at -20°C. This conjugate requires dilution in an appropriate buffer prior to use. The diluted conjugate should not be stored; discard after use.

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General Procedure

Intended Use: These reagents have been prescreened to allow construction of a sandwich enzyme immunoassay (EIA) for the specific and quantitative measurement of cytokines. The enclosed procedures provide general guidelines for EIA preparation. All investigators should be aware that antibody concentrations, sample type, sample matrix, EIA procedure (time, temperature, etc.) can all affect the performance characteristics of the assay and must be optimized. These reagents are intended **for research use only. They are not to be used for diagnostic purposes.**

Plate Coating

1. Dilute coating antibody in **Coating Buffer** to the concentration recommended on the accompanying CytoSets™ information sheet. Refer to vial label for concentration of coating antibody.
2. Add 100 µL of diluted Coating Antibody per well to polystyrene microplates. (e.g., Dynex Immulon 2 HB, Catalog Number: 6506 or Nunc Maxisorp, Catalog Number: 468667).
3. Cover the plates and incubate overnight (12 to 18 hours) at 2 - 8°C.
4. Aspirate the coating antibody from the wells and tap on absorbent paper to remove excess liquid.
5. Add 300 µL of **Blocking Solution** (see Table 1) to each well. Cover the plates and incubate for at least 2 hours at room temperature. If not used immediately, plates may be stored sealed for up to 5 days at 4°C in **Blocking Solution**.
6. Prior to use, aspirate the **Blocking Solution** from the wells and tap on absorbent paper to remove excess liquid.
7. Wash the microplate 3 to 6x with 400 µL per well of **Wash Solution** (see Table 1) and tap on absorbent paper to remove all excess liquid after the final wash. Do not allow wells to dry completely at any time.

ELISA Method

1. Add Standards and Samples to Microplate.
   - Dilute standards and samples in **Standard Diluent/Assay Buffer** (see Table 1) or in the assay matrix most relevant to your samples (e.g., cell culture medium containing 10% fetal calf serum).
   - Add 100 µL of standards, samples and controls to appropriate wells, in duplicate. Controls can include a reagent blank (Zero Standard Control) and a substrate blank.
   - **Important Note:** Some assays may require separate incubation of standards, samples and controls prior to addition of the biotinylated detection antibody (Step #2). Refer to the accompanying CytoSets™ information sheet for specific instructions under standard incubation recommendations.

2. Add biotinylated detection antibody
   - Dilute biotinylated detection antibody in **Standard Diluent/Assay Buffer** (see Table 1) to the concentration recommended on the accompanying CytoSets™ information sheet.
   - Add 50 or 100 µL of diluted detection antibody (as recommended on CytoSets™ information sheet) to each well except chromogen blank, cover the plate and incubate for the time and temperature recommended on the accompanying CytoSets™ information sheet.
   - Aspirate solution from wells.
   - Wash the microplate 3 to 6x with 400 µL per well of **Wash Solution** (see Table 1) and tap on absorbent paper to remove all excess liquid after the final wash.

3. Add Streptavidin-Horseradish Peroxidase Conjugate
   - Dilute Streptavidin-HRP conjugate according to the manufacturer's instructions. Streptavidin-HRP may be diluted in **Standard Diluent/Assay Buffer** (see Table 1) if not otherwise specified by the manufacturer.
   - Add 100 µL of diluted Streptavidin-HRP per well, cover the microplate and incubate at room temperature for 15 to 45 minutes. (Note: Dilution and incubation time will vary depending on manufacturer and lot).
   - Aspirate solution from wells.
   - Wash the microplate 3 to 6x with 400 µL per well of **Wash Solution** (see Table 1) and tap on absorbent paper to remove all excess liquid after the final wash.
4. Add Substrate
   - Prepare TMB (tetramethylbenzidine) substrate according to manufacturer's instructions.
   - Add 100 µL of TMB to each well and incubate in the dark at room temperature for 10 to 60 minutes (generally 30 minutes) according to manufacturer's instructions. (Note: Incubation time will vary depending on manufacturer and lot).
   - Stop the reaction by addition of 50 or 100 µL of Stop Solution to each well according to manufacturer's instructions.

5. Read microplate
   - Read microplate at 450 nm within 30 minutes of adding Stop Solution (reference filter: 630 or 650 nm).
   - Calculate the average optical density at 450 nm for all Standards, Controls and Samples. Construct a standard curve by plotting each Standard optical density (ordinate) vs. the Standard concentration (abscissa) on semi-log graph paper. For plate readers with automated standard curve calculation capability, a log-log or four parameter curve fit algorithm may provide the best curve fit.
   - Determine the concentration of each unknown sample from the standard curve.

For lot specific information refer to CytoSets™ Information Sheet

Table 1: Suggested Solution Formulations

ALL SOLUTIONS MUST BE PREPARED JUST PRIOR TO USE

Solution	Formulation
Coating Buffer A	8.0 g NaCl 1.42 g Na_2HPO_4•$2H_2O$ 0.2 g KH_2PO_4 0.2 g KCl q.s. to 1 liter with distilled H_2O, pH 7.4
Coating Buffer B	4.3 g $NaHCO_3$ 5.3 g Na_2CO_3 q.s. to 1 liter with distilled H_2O, pH 9.4
Blocking Solution	8.0 g NaCl 1.42 g Na_2HPO_4•$2H_2O$ 0.2 g KH_2PO_4 0.2 g KCl 5.0 g bovine serum albumin (fraction V) q.s. to 1 liter with distilled H_2O, pH 7.4
Standard Diluent/Assay Buffer	8.0 g NaCl 1.42 g Na_2HPO_4•$2H_2O$ 0.2 g KH_2PO_4 0.2 g KCl 5.0 g bovine serum albumin (fraction V) 1 mL Tween 20 q.s. to 1 liter with distilled H_2O, pH 7.4
Wash Buffer	9.0 g NaCl 1 mL Tween 20 q.s. to 1 liter with distilled H_2O, pH 7.4
Stop Solution	1.8 N H_2SO_4

***This procedure contains only recommendations. Investigators are advised to determine optimal buffer formulations, concentrations and incubation times for individual applications.**

Note: This procedure has not been optimized for use with serum or plasma samples.

Accessory reagents available from BioSource International Inc./Biosource Europe:

Streptavidin-HRP:

41.000.03	Streptavidin-HRP, lyophilized, 0.9 µg/vial
SNN2004	Streptavidin-HRP ELISA grade, liquid, 1mg/1mL

TMB (tetramethylbenzidine):

45.011.03	TMB Concentrate, liquid, 1mL
45.014.01	TMB Substrate Buffer, liquid, 21 mL
SB01	TMB Liquid, Read-to-use, 25 mL