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IFN-gamma PeliPair Reagent Set

Catalog No: M9333 **Size:** 19 x 96 tests

Introduction

PeliPairs are sets of basal ELISA reagents for the design of enzyme immunoassays to quantify human cytokines in cell culture supernatants.

Contents of the Reagents Set

Component	# of Vials	Volume	Cap Color
Coating Antibody	5	375 µl	Red
Standard	5	See vial	-
Biotinylated antibody	5	375 µl	Yellow

Storage of Reagents

The Pelipairs ELISA Reagents Set should be stored at temperatures between -18°C to -32°C. The performance of the reagents is guaranteed until the expiration date shown on the label. Liquid or freeze-dried standard (kit dependent), should be stored frozen after use (-18° to -32°C, preferably < -80°C).

Precautions

- The PeliPair Reagent Set is intended *for research purposes only*.
- Only use the reagents supplied with the kit. Never mix reagents from different kit lots.
- Handle all plasma and serum samples with care to prevent transmission of blood-borne infections.
- Sodium azide will inactivate HRP, so do not use sodium azide-containing solutions, nor add sodium azide to the reagents.
- We advise the use of NUNC MAXISORP microtiter plates. The supplied reagents have only been tested and approved for use on this brand of microtiter plates. The efficacy on other types of microtiter plates is not guaranteed.
- Centrifuge all vials before use (1 min at 3000 x g).
- Wells should not stand uncovered or allowed to dry between incubation steps.

Cell Sciences, Inc.
480 Neponset Street
Building 12A
Canton, MA 02021

Toll Free: 888 769-1246
Phone: 781 828-0610
Fax: 781 828-0542

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

General Assay Protocol

1) Coating

- Dilute coating antibody at least 1:100 in coating buffer (see **Recommended Buffers and Solutions**).
- Transfer 100 µl to all wells of the microtiter plate.
- Cover plate with sealer and incubate overnight at 2-4°C.

2) Wash

Wash microtiter plate(s) five times with PBS (see **Recommended Buffers and Solutions**). After the final wash the wells should be dry.

3) Blocking

- Block plates with 200 µl of blocking buffer (see **Recommended Buffers and Solutions**) per well.
- Cover plate with sealer and incubate for 1 hour at room temperature (18-25°C).

4) Wash

Wash microtiter plate(s) five times with washing buffer (see **Recommended Buffers and Solutions**).

5) Incubation with Standard and Samples

- Dilute standard and samples in dilution buffer (see **Recommended Buffers and Solutions**).
- Transfer 100 µl into the appropriate duplicate wells.
- Cover plate with sealer and incubate for 1 hour at room temperature (18-25°C).

6) Wash

Wash microtiter plate(s) five times with washing buffer.

7) Incubation with Biotin Conjugate

- Dilute biotinylated antibody at least 1:100 in dilution buffer (see **Recommended Buffers and Solutions**).
- Transfer 100 µl into the appropriate duplicate wells.
- Cover plate with sealer and incubate for 1 hour at room temperature (18-25°C)

8) Wash

Wash microtiter plate(s) five times with washing buffer.

9) Incubation of Streptavidin-HRP Conjugate

- Dilute streptavidin-HRP conjugate in dilution buffer (see **Recommended Buffers and Solutions**).
- Transfer 100 µl in duplicate into the appropriate wells.
- Cover plate with sealer and incubate for 30 minutes at room temperature (18-25°C).

10) Wash

Wash microtiter plate(s) five times in washing buffer.

11) Substrate Incubation

- Prepare substrate solution (see **Recommended Buffers and Solutions**).
- Transfer 100 µl of substrate solution to all wells.
- Incubate at room temperature (18-25°C) in the dark.

Note: the speed of enzymatic color development is influenced by many factors including temperature and quality of the used substrate.

12) Stop Enzymatic Reaction and Plate Read-Out

Add 100 µl of stop solution to each well and measure absorbance within 30 minutes at the appropriate wavelength.

Recommended Buffers and Solutions

- **Coating buffer**: 100 mM Carbonate/bicarbonate buffer, pH 9.6
- **PBS**: 10 mM Phosphate buffered saline, pH 7.3
- **Blocking buffer**: PBS with 1-5% protein
- **Washing buffer**: PBS with 0.005 - 0.01 % TWEEN-20
- **Dilution buffer**: High Performance ELISA buffer
- **Standard**: Dilute stock standard in dilution buffer to obtain highest standard point (optimized standard curve range for IFN-gamma is 2.0-500 pg/ml) and prepare serial dilutions.
- **Streptavidin-HRP conjugate**: Streptavidin-poly-HRP, dilute 1:5,000 - 1:10,000
- **Substrate solution**: TMB (3,5,3',5'-tetramethylbenzidine)
- **Stop solution**: 1.8 M H_2SO_4

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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