Sanquin

PeliPair™ reagent set

1824 tests (19 plates)

Antibody pair and standard for quantitative determination
of human cytokines by enzyme immunoassay.

PRODUCT INFORMATION

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I. INTRODUCTION

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

II. CONTENTS OF THE REAGENTS SET

Each Pelipair Reagent Set contains material sufficient for at least 1824 tests (19 plates).

Reagents provided:

Component	No.of vials	Volume	Cap colour
Coating antibody	5	375 µl	Red
Standard	5	information on vial	-
Biotinylated antibody	5	375 µl	Yellow

III. STORAGE AND STABILITY

The Pelipairs ELISA Reagents Set should be stored between –18°C to -32°C.
The performance of the reagents is guaranteed until the expiration date shown on the labels.

Liquid or freeze-dried standard (kit dependent), should be stored frozen after use (-18° to -32°C, preferably < -70°C).

IV. PRECAUTIONS FOR USE

1) The PeliPair Reagent Set is intended *for research purposes only*.
2) Only use the reagents supplied with the kit. Never mix reagents from different kit lots.
3) Handle all plasma and serum samples with care to prevent transmission of blood-borne infections.
4) Sodium azide will inactivate HRP, so do not use sodium azide-containing solutions, nor add sodium azide to the reagents.
5) 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.
6) Centrifuge all vials before use (1 min at 3000 x g).
7) Wells should not stand uncovered or allowed to dry between incubation steps.

V. GENERAL ASSAY PROTOCOL

1) **Coating**

Dilute coating antibody at least 1:100 in **coating buffer** (see section VI).
Transfer 100 µl to all wells of the microtiter plate.
Cover plate with sealer and **incubate overnight at 2-8°C**.

2) **Washing**

Wash microtiter plate(s) five times with **PBS** (see section VI).
After the final wash the wells should be dry.

3) **Blocking**

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

4) **Washing**

Wash microtiter plate(s) five times with **washing buffer** (see section VI).

5) **Incubation with standard and samples**

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

6) **Washing**

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 section VI).
Transfer 100 µl into the appropriate duplicate wells.
Cover plate with sealer and incubate for 1 **hour at room temperature (18-25°C).**

8) **Washing**

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

9) **Incubation of streptavidin-HRP conjugate**

Dilute streptavidin-HRP conjugate in **dilution buffer** (see section VI).
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) **Washing**

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

11) **Substrate incubation**

Prepare substrate solution (see section VI).
Transfer 100 µl of substrate solution to all wells.
Incubate at room temperature (18-25°C) in the dark.
Note: the speed of enzymatic colour 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.

VI. RECOMMENDED BUFFERS & SOLUTIONS

NB. Instead of making all the buffers and solutions by yourself, you can also use our PeliKine Toolset (Cat.No. M1980).

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 (Cat.No. M1941)
Washing buffer* : PBS with 0.005 - 0.01 % TWEEN-20
Dilution buffer : High Performance ELISA buffer (HPE, Cat.No. M1940)
Standard : Dilute stock standard in dilution buffer to obtain highest standard point (check table below) and prepare serial dilutions
Streptavidin-HRP conjugate : Streptavidin-poly-HRP, dilute 1:5000 - 1:10.000 (Cat.No. M2032)
Substrate solution* : TMB (3,5,3',5'-tetramethylbenzidine)
Stop solution* : 1.8 M H_2SO_4

*Components of the PeliKine Toolset

Available PeliPairs:

Cat.No.	Human cytokine	Optimized standard curve range (pg/ml)
M9310	**Interleukin 10**	**1.2 - 300**
M9313	**Interleukin 13**	**0.5 - 125**
M9314	**Interleukin 4**	**0.6 - 450**
M9316	**Interleukin 6**	**0.6 - 450**
M9318	**Interleukin 8**	**1.0 - 240**
M9333	**Interferon γ**	**2.0 - 500**
M9334	**Interleukin 1β**	**0.4 - 300**