Interleukin 6
matched pair for ELISA

Cat.No: **21339068** (contains 2 vials with Cat.No 21339061 and 21339062)

I. Introduction
ImmunoTools matched pairs reagents are design for enzyme immunoassays

II. Contents of the matched pair SET
Each ImmunoTools matched pair Set contains material sufficient for 480 -1920 tests (**5** to **20** plates) dependent from the sensitivity you need.

Reagents provided:
- **Coating antibody** (yellow cup): 100µg, PBS, 0.09 % sodium azide
- **Biotinylated detector** (pink cap): 50µg, PBS, 1 % BSA, 0.09 % sodium azide

III. Storage and stability
The ImmunoTools matched pairs should be stored at 4 °C. The performance of the reagents is guaranteed until the expiration date shown on the label.

IV. Precautions for use
1) The ImmunoTools matched pair is intended *for research purposes only*.
2) Centrifuge all vials before use (1 min at 3000 x g).
3) Sodium azide will inactivate HRP, so do not use sodium azide-containing solutions, nor add sodium azide to the reagents. Since both reagents contained sodium azide, please take care that the plates are washed five times with washing buffer you transfer HRP-streptavidin
4) Wells should not stand uncovered or allowed to dry between incubation steps.

V. GENERAL ASSAY PROTOCOL
1) **Coating**
Dilute coating antibody between 1:500 and 1:2000 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)**.

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

7) **Incubation with biotin conjugate**
Dilute biotinylated antibody between 1:500 and 1:2000 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) **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

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 (e.g. BSA, HSA, ovalbumin, skimmed milk)
Washing buffer		PBS with 0.005 - 0.01 % TWEEN-20
Dilution buffer		Culture medium with 5-10 % FCS
	or	PBS with 1 % BSA and 0.05 % TWEEN-20
Standard		Dilute stock standard in dilution buffer to obtain highest standard point and prepare serial dilutions
Streptavidin-HRP conjugate		
Substrate solution		TMB (3,5,3',5'-tetramethylbenzidine)
	or	OPD (*o*-Phenylenediaminedihydrochloride)
	or	ABTS (2,2'-Aminobis(3-ethylbenzthiazolinesulfonic acid)
Stop solution		Substrate dependent: H_2SO_4 / HCl / $C_2H_2O_4$ (oxalic acid)

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