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Data Sheet

Colorimetric Human IL-6 ELISA Detection Kit

Catalog #79820-1
Size: 96 reactions

DESCRIPTION: Interleukin-6 is a cytokine produced in response to infections or tissue damage that plays an important role in inflammation, immune response and hematopoiesis. The *Colorimetric Human IL-6 Detection Kit* is designed for detecting and quantifying human interleukin-6 in cell culture medium. This kit comes in a convenient 96-well format, with enough capturing and detection antibodies for IL-6, streptavidin-labeled HRP, blocking buffer, IL-6 standard, and colorimetric HRP substrate for a 96-well plate. Only a few simple steps on a microtiter plate are required for the assay. First, the capturing antibody is coated on a 96-well plate. Next, samples containing IL-6 are incubated on the coated plate followed by detecting the captured IL-6 with the detection antibody. Finally, the plate is treated with streptavidin-HRP followed by addition of a colorimetric HRP substrate to produce color, which can then be measured using a UV/Vis spectrophotometer microplate reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
	Interleukin-6 capturing antibody (1 mg/ml)	10 µl	-80°C	***(Avoid freeze/ thaw cycles!)***
	Interleukin-6 detection antibody, biotinylated	5 µl	-80°C	
	Human IL-6 standard (1 µg/ml)	20 µl	-80°C	
80611	Streptavidin-HRP	5 µl	+4°C	
79743	Blocking Buffer 3	50 ml	+4°C	
79651	Colorimetric HRP substrate	10 ml	+4°C	
	Transparent 96-well microplate	1	+4°C	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

PBS (Phosphate-buffered saline)
PBST (PBS with 0.05% Tween-20)
1N HCl (aqueous)
Rotating or rocker platform
UV/Vis spectrophotometer microplate reader capable of reading absorbance at 450 nm*

**Alternatively, a spectrophotometer reading at 650 nm may be used without adding 1N HCl, but sensitivity of the assay will be reduced*.

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APPLICATIONS: This kit is useful for cytokine detection in cell culture medium.

STABILITY: One year from date of receipt when stored as directed.

REFERENCE:

Tanaka T., *et al*. IL-6 in Inflammation, Immunity and Disease. *Cold Spring Harbor Perspect. Biol.* 2014. **6(10)**: 1-16.

ASSAY PROTOCOL:
All samples and standards should be tested in duplicate.

Step 1) Coating the plate with capturing Ab:

1) Thaw **capturing Ab** on ice. Upon first thaw, *briefly* spin tube containing **capturing Ab** to recover the full contents of the tube.

2) Dilute **capturing Ab** to 2 ng/µl in PBS.

3) Add 50 µl of diluted **capturing Ab** solution to each well and incubate overnight at 4°C. (Remaining **capturing Ab** can be stored at 4°C)

   **After overnight coating, it is highly recommended that all remaining steps are completed the following day to obtain optimal sensitivity.*

4) After the overnight incubation, decant to remove the solution. Wash the plate 2 times with 200 µl/well of PBS with 0.05% Tween-20 (PBST). Tap plate onto clean paper towels to remove liquid.

5) Block wells by adding 200 µl of **Blocking Buffer** to each well. Incubate for 1 hour at room temperature. Decant to remove the blocking buffer and wash the plate 2 times with 200 µl/well of PBST. Tap plate onto clean paper towels to remove liquid.

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Step 2) Assay Procedure:

1) Serially dilute the **human IL-6 standard** in **Blocking Buffer** at 2-fold dilutions from 500 pg/ml to 15 pg/ml. Aliquot any remaining **human IL-6** into single use aliquots. Immediately store remaining undiluted protein in aliquots at -80°C.

2) Prepare the sample by diluting in the **Blocking Buffer**. The detection range of the *Colorimetric Human IL-6 Detection Kit* is 15 pg/ml – 500 pg/ml (**Figure 1**). Roughly estimate the amount of human IL-6 in the sample and dilute it accordingly.

3) Add 50 µl of the **human IL-6 standard** or diluted samples to each well and incubate the plate for 2 hours at room temperature. Include a couple wells with just **Blocking Buffer** ("Blank wells" for use as a negative control.

4) After 2 hours incubation, decant to remove the solution and wash the plate 2 times with 200 µl/well of PBST. Tap plate onto clean paper towels to remove liquid.

5) Dilute **biotinylated-detection Ab** 1:1,000 in the Blocking Buffer, and add 50 µl to the wells. Incubate the plate for 1 hour at room temperature.

6) After 1-hour incubation, decant to remove the solution and wash the plate 3 times with 200 µl/well of PBST. Tap plate onto clean paper towels to remove liquid.

7) Dilute **Streptavidin-HRP** 1:1,000 in the Blocking Buffer, and add 50 µl to the wells. Incubate the plate for 30 minutes at room temperature.

8) After 30 minutes incubation, decant to remove the solution and wash the plate 5 times with 200 µl/well of PBST. Tap plate onto clean paper towels to remove liquid

9) Add 100 µl of the **Colorimetric HRP substrate** to each well and incubate the plate at room temperature until blue color is developed in the positive control well. This usually takes several minutes. The optimal incubation time may vary, and should be determined empirically by the user.

10) After the blue color is developed, add 100 µl of 1 M HCl to each well. Read the absorbance at 450 nm using UV/Vis spectrophotometer microplate reader. The blank wells should exhibit an absorbance of ~ 0.05 at 450 nm. *Alternatively, the plate may be read at 650 nm without adding 1N HCl, but the Signal-to-Background ratio will be decreased*.

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Example of Detection Results:

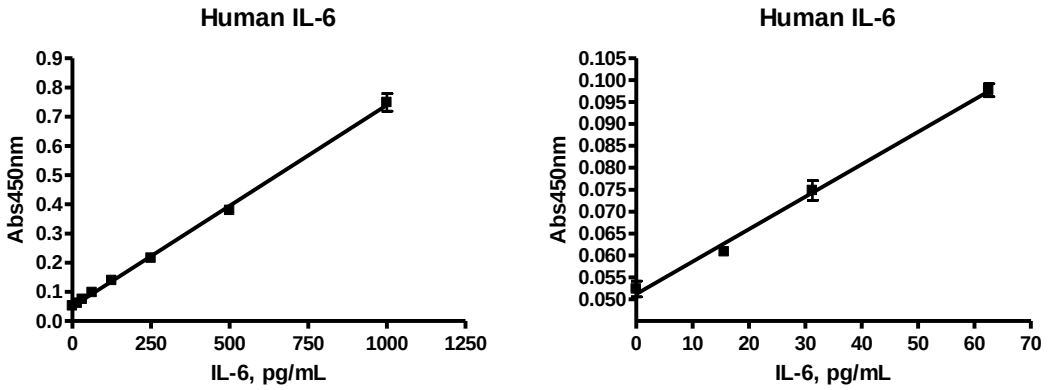

Figure 1. Detection of the human IL-6 (left:15 pg/ml – 1000 pg/ml, right: 15 pg/ml – 60 pg/ml) standard using the *Colorimetric™ Human IL-6 Detection Kit.* *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	**Catalog #**	**Size**
Human Interleukin-6	90196-B	20 µg
Mouse Interleukin-6	90197-A	2 µg
Mouse Interleukin-6	90197-B	10 µg
Streptavidin-HRP	80611	100 µl
Blocking Buffer 3	79743	50 ml

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Colorimetric signal of positive control reaction is weak	Antibodies have lost activity	Antibodies and IL-6 standard may lose activity upon repeated freeze/thaw cycles. Use fresh protein. Store proteins in single-use aliquots. Increase time of incubation. Increase protein or antibody concentration.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity.
	Colorimetric HRP substrate was not incubated long enough	Increase the amount of time that the colorimetric HRP substrate is incubated in the wells. Avoid azides.
Colorimetric signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
	Signal is out of range of detection (too high)	Decrease the amount of time that the colorimetric HRP substrate is incubated in the wells
Background (signal to noise ratio) is high	Insufficient washes or blocking	Be sure to include blocking steps after wash steps. Increase number of washes. Increase wash volume. Increase Tween-20 concentration to 0.1% in TBST. Be sure to dilute Streptavidin-HRP in blocking buffer, not assay buffer.
	Sample solvent is inhibiting the protein	Run negative control assay including solvent. Maintain DMSO level at <1%. Increase time of protein incubation.
	Results are outside the linear range of the assay	Use different concentrations of IL-6 standard to create a standard curve.