IL-6 (pig)
IL-6 (pig) Enzyme Immunoassay kit #A05410.96 wells

For research laboratory use only
Not for human diagnostic use

This assay has been developed & validated by Bertin Pharma
This kit contains:

<table>
<thead>
<tr>
<th>Designation</th>
<th>Colour of cap</th>
<th>Item #</th>
<th>Quantity per kit</th>
<th>Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 precoated 96-well Strip Plate</td>
<td>Blistor with zip</td>
<td>A08410.1 ea</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Streptavidin Poly_HRP Tracer</td>
<td>Green</td>
<td>A04110.100 dtm</td>
<td>1 Liquid</td>
<td></td>
</tr>
<tr>
<td>IL-6 (pig) Biotin-labelled Antibody</td>
<td>Red</td>
<td>A03110.100 dtm</td>
<td>1 Liquid</td>
<td></td>
</tr>
<tr>
<td>IL-6 (pig) Standard</td>
<td>Blue with red septum</td>
<td>A06410.1 ea</td>
<td>2 Lyophilised</td>
<td></td>
</tr>
<tr>
<td>Poly_HRP EIA Buffer</td>
<td>Grey / Blue</td>
<td>A07410.1 ea</td>
<td>1 Lyophilised</td>
<td></td>
</tr>
<tr>
<td>Wash Buffer</td>
<td>Silver</td>
<td>A17000.1 ea</td>
<td>1 Liquid</td>
<td></td>
</tr>
<tr>
<td>Tween 20</td>
<td>Transparent</td>
<td>A12000.1 ea</td>
<td>1 Liquid</td>
<td></td>
</tr>
<tr>
<td>HRP Substrate Solution</td>
<td>Black</td>
<td>A09034.100 dtm</td>
<td>1 Liquid</td>
<td></td>
</tr>
<tr>
<td>HRP Stop Solution</td>
<td>Yellow</td>
<td>A24110.100 dtm</td>
<td>1 Liquid</td>
<td></td>
</tr>
<tr>
<td>Instruction Booklet</td>
<td>-</td>
<td>A11410.1 ea</td>
<td>1 -</td>
<td></td>
</tr>
<tr>
<td>Well cover Sheet</td>
<td>-</td>
<td>-</td>
<td>1 -</td>
<td></td>
</tr>
</tbody>
</table>

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 37 samples in duplicate.
Precaution for use

Users are recommended to carefully read all instructions for use before starting work.

Each time a new pipette tip is used, aspirate a sample or reagent and expel it back into the same vessel. Repeat this operation two or three times before distribution in order to equilibrate the pipette tip.

- For research laboratory use only
- Not for human diagnostic use
- Do not pipet liquids by mouth
- Do not use kit components beyond the expiration date
- Do not eat, drink or smoke in area in which kit reagents are handled
- Avoid splashing

HRP Stop Solution and HRP Substrate Solution are harmful solutions. To avoid any contact, wear eye, hand, face and clothing protection when handling these.

Wearing gloves, laboratory coat and glasses is recommended when assaying kit materials and samples.

Temperature

Unless otherwise specified, all the experiments are done at room temperature (RT), that is around +20°C. Working at +25°C or more affects the assay.

Background

Interleukin-6 (IL-6)

IL-6 is a protein of 212 amino acids [1]. It’s a pleiotropic cytokine which has an impact on the regulation of immune and nonimmune events [2]. It is produced by the monocytes and macrophages in response to other inflammatory cytokines like Interleukin-11 (IL-11) and Tumor Necrosis Factor-beta (TNF-β).

IL-6 acts on immune and nonimmune cells: it helps to differentiate T-cells from B-cells but it is also vital for the development of blood cells (white cells, red cells or platelets).

Due to these target cells, IL-6 is an activator of the immune system and the inflammation [3]. It also controls the metabolism by action on adipocytes [4], the bone metabolism and the pain.

A dysregulation of IL-6 production induces many disorders such as autoimmune diseases, inflammation, diabetes [2] or cancer.
**Principle of the assay**

This Enzyme Immunometric Assay (EIA/ELISA) is based on a sandwich technique. The wells of the plate supplied are coated with a polyclonal antibody specific to IL-6 (pig).

IL-6 (pig) introduced into the wells (standard or sample) will be bound by the polyclonal antibody coated on the plate and is then detected by a second polyclonal antibody tagged with biotin also specific for IL-6 (pig).

The two antibodies then form a sandwich by binding on different parts of IL-6 (pig). The sandwich is immobilised on the plate so reagents in excess may be washed away.

The immunological complex is revealed by the interaction between biotin and streptavidin labelled with HRP (Tracer).

The concentration of IL-6 (pig) is determined by measuring the enzymatic activity of immobilized Tracer using TMB. The Tracer acts on TMB to form a yellow compound after the reaction has been stopped.

The intensity of the colour, which is determined by spectrophotometry at 450 nm, is proportional to the amount of IL-6 (pig) present in the well during the immunological incubation.
Materials and equipment required

In addition to standard laboratory equipment, the following material is required:

For the assay:

- Precision micropipettes (20 to 1000 μL)
- Spectrophotometer plate reader (450 nm filter)
- Microplate washer (or washbottles)
- Orbital microplate shaker
- Multichannel pipette and disposable tips 30-300 μL
- UltraPure water #A07001.1L
- Polypropylene tubes

Water used to prepare all ELISA reagents and buffers must be UltraPure, deionized & free from organic contaminant traces.
Do not use distilled water, HPLC-grade water or sterile water.

UltraPure water may be purchased from Bertin Pharma (item #A07001.1L).

Sample collection and preparation

This assay may be used to measure IL-6 (pig).

It is the responsibility of the user to check the compatibility of the assay with the study matrix.

General precautions

- All samples must be free from organic solvents prior to assay.
- Samples should be assayed immediately after collection or should be stored at -20°C or at -80°C.
## Reagent preparation

All reagents need to be brought to room temperature (around +20°C) prior to the assay.

### Poly_HRP EIA Buffer

Reconstitute Poly_HRP EIA Buffer #A07410 with 25 mL of UltraPure water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion. **Stability at 4°C: 1 month.**

Before use, filter the Buffer on 0.22 μm filter.

### IL-6 (pig) Standard

Reconstitute the IL-6 (pig) Standard vial #A06410 with 1 mL of UltraPure water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion. **Stability at 4°C: within the day.**

<table>
<thead>
<tr>
<th>Standard</th>
<th>Volume of Standard</th>
<th>Volume of Poly_HRP EIA Buffer</th>
<th>Standard concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>-</td>
<td>-</td>
<td>10.00 ng/mL</td>
</tr>
<tr>
<td>S2</td>
<td>500 μL of S1</td>
<td>500 μL</td>
<td>5.00 ng/mL</td>
</tr>
<tr>
<td>S3</td>
<td>500 μL of S2</td>
<td>500 μL</td>
<td>2.50 ng/mL</td>
</tr>
<tr>
<td>S4</td>
<td>500 μL of S3</td>
<td>500 μL</td>
<td>1.25 ng/mL</td>
</tr>
<tr>
<td>S5</td>
<td>500 μL of S4</td>
<td>500 μL</td>
<td>0.62 ng/mL</td>
</tr>
<tr>
<td>S6</td>
<td>500 μL of S5</td>
<td>500 μL</td>
<td>0.31 ng/mL</td>
</tr>
<tr>
<td>S7</td>
<td>500 μL of S6</td>
<td>500 μL</td>
<td>0.16 ng/mL</td>
</tr>
<tr>
<td>S8</td>
<td>500 μL of S7</td>
<td>500 μL</td>
<td>0.08 ng/mL</td>
</tr>
</tbody>
</table>

**Stability of diluted antibody at +4°C: within the day.**

### IL-6 (pig) Biotin-labelled Antibody

The IL-6 Biotin-labelled Antibody is supplied concentrated 10 times. Calculate the volume needed (number of wells multiplied by 0.1 mL) and then dilute the IL-6 Biotin-labelled Antibody solution #A03410 with the appropriate volume of Poly_HRP EIA Buffer.

**Example:** for 40 wells you need 4 mL of IL-6 biotin-labelled Antibody (40 x 0.1 mL), add 0.4 mL of IL-6 Biotin-labelled Antibody in 3.6 mL of Poly_HRP EIA Buffer.

**Stability of diluted antibody at +4°C: within the day.**
Assay procedure

It is recommended to perform the assays in duplicate following the instructions hereafter.

Plate preparation

Prepare the Wash Buffer as indicated in the reagent preparation section.
Open the plate packet and select the sufficient strips for your assay. Put unused strips back in the zip lock bag with the desiccant pocket and properly close it.
*Stability at +4°C: 1 month.*

Rinse each well 5 times with Wash Buffer (300 μL/well).

Just before distributing the reagents and samples, remove the buffer from the wells by inverting the plate and shaking out the last drops on a paper towel.

Plate set-up

A plate set-up is suggested on the following page. The contents of each well may be recorded on the template sheet provided at the end of this technical booklet.

Pipetting the reagents

All samples and reagents must reach room temperature prior to performing the assay.

Use different tips to pipet all the reagents.

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Wash Buffer

Dilute 2 mL of concentrated Wash Buffer #A17000 with 800 mL of UltraPure water. Add 400 μL of Tween 20 #A12000. Use a magnetic stirring bar to mix the content. 
*Stability at +4°C: 1 month.*
Before pipetting, equilibrate the pipette tips in each reagent. Do not touch the liquid already in the well when expelling with the pipette tip.

- **Poly_HRP EIA Buffer**
  Dispense 100 µL to NSB wells.

- **IL-6 (pig) Standard/Sample**
  Dispense 100 µL of each of the eight standards (S8 to S1) in duplicate to appropriate wells.

Start with the lowest concentration standard (S8) and equilibrate the tip in the next higher standard before pipetting.

**Incubating the plate**
Cover the plate with the cover sheet and incubate 60 minutes at room temperature, shaking at 300 rpm.

**Washing the plate**
Rinse each well 5 times with the Wash Buffer (300 µL/well). Just before distributing reagents, remove the buffer from the wells by inverting the plate and shaking out the last drops on a paper towel.

**Pipetting the reagents**
- **IL-6 (pig) Biotin-labelled antibody**
  Dispense 100 µL to each well, except Blank (Bk) wells.

**Incubating the plate**
Cover the plate with the cover sheet and incubate 60 minutes at room temperature, shaking at 300 rpm.

**Washing the plate**
Rinse each well 5 times with the Wash Buffer (300 µL/well). Just before distributing reagents, remove the buffer from the wells by inverting the plate and shaking out the last drops on a paper towel.
**Pipetting the reagents**

- **Streptavidin Poly_HRP Tracer**
  Dispense 100 μL to each well, except Blank (Bk) wells.

**Incubating the plate**

Cover the plate with the cover sheet and incubate 30 minutes at room temperature, shaking at 300 rpm.

**Developing and reading the plate**

- Rinse each well 5 times with 300 μL of Wash Buffer. At the end of the last washing step, empty the plate and blot the plate on a paper towel to discard any trace of liquid.
- Add 100μL of HRP Substrate Solution to each well. Incubate the plate in the dark at room temperature without shaking. For the time, look at the lot specific Quality Control Sheet (QCS). In general, revelation time is 10 min.
- Add 100μL of HRP Stop Solution to each well.
- Wipe the bottom of the plate with a paper towel, and make sure that no liquid has splashed outside the wells.
- Read the plate at 450 nm (yellow color).

### Enzyme Immunoassay Protocol (volumes are in μL)

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>NSB</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly_HRP EIA Buffer</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
</tbody>
</table>

Cover plate, incubate 60 minutes at room temperature under orbital shaking at 300 rpm
Wash strips 5 times with 300 μL/well
Discard liquid from the wells & dry on absorbent paper

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>NSB</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin-labelled Antibody</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Cover plate, incubate 60 minutes at room temperature under orbital shaking at 300 rpm
Wash strips 5 times with 300 μL/well
Discard liquid from the wells & dry on absorbent paper

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>NSB</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptavidin Poly_HRP Tracer</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Cover plate, incubate 30 minutes at room temperature under orbital shaking at 300 rpm
Wash strips 5 times with 300 μL/well
Discard liquid from the wells & dry on absorbent paper

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>NSB</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRP Substrate Solution</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Incubate the plate in the dark without agitation

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>NSB</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRP Stop Solution</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Read the plate at 450 nm
Data analysis

Make sure that your plate reader has subtracted the absorbance readings of the blank wells (absorbance of HRP Substrate Solution alone) from the absorbance readings of the rest of the plate. If it is not the case, please do it.

- Calculate the average absorbance for each NSB, standard and sample.
- For each standard, plot the absorbance on y axis versus the concentration on x axis. Draw a best-fit line through the points.
- To determine the concentration of your samples, find the absorbance value of each sample on the y axis.
- Read the corresponding value on the x axis which is the concentration of your unknown sample.
- Samples with a concentration greater than 10 ng/mL should be re-assayed after dilution in Poly_HRP EIA Buffer.
- Most plate readers are supplied with a curve-fitting software capable of graphing these data (4-parameter logistic fit 4PL). If you have this type of software, we recommend using it. Refer to it for further information.

Acceptable range

- NSB absorbance ≤0.150 A.U.
- Limit of detection ≤0.08 ng/mL
Typical results

The following data are for demonstration purpose only. Your data may be different and still correct.

These data were obtained using all reagents as supplied in this kit under the following conditions: 10 minutes developing at room temperature, reading at 450 nm. A 4 parameter logistic fitting was used to determine the concentrations.

<table>
<thead>
<tr>
<th>Standard</th>
<th>IL-6 (pig) ng/mL</th>
<th>Absorbance A.U.</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>10.00</td>
<td>3.067</td>
</tr>
<tr>
<td>S2</td>
<td>5.00</td>
<td>2.234</td>
</tr>
<tr>
<td>S3</td>
<td>2.50</td>
<td>1.373</td>
</tr>
<tr>
<td>S4</td>
<td>1.25</td>
<td>0.803</td>
</tr>
<tr>
<td>S5</td>
<td>0.62</td>
<td>0.436</td>
</tr>
<tr>
<td>S6</td>
<td>0.31</td>
<td>0.280</td>
</tr>
<tr>
<td>S7</td>
<td>0.16</td>
<td>0.167</td>
</tr>
<tr>
<td>S8</td>
<td>0.08</td>
<td>0.120</td>
</tr>
<tr>
<td>NSB</td>
<td>0.00</td>
<td>0.068</td>
</tr>
</tbody>
</table>

Typical IL-6 (pig) standard curve

**Characteristics**

**Cross-reactivity**

<table>
<thead>
<tr>
<th>Species</th>
<th>Cross-reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant IL-6 (bovine)</td>
<td>+</td>
</tr>
<tr>
<td>Recombinant IL-6 (canine)</td>
<td>++</td>
</tr>
<tr>
<td>Recombinant IL-6 (chicken)</td>
<td>-</td>
</tr>
<tr>
<td>Recombinant IL-6 (equine)</td>
<td>+</td>
</tr>
<tr>
<td>Recombinant IL-6 (feline)</td>
<td>++</td>
</tr>
</tbody>
</table>

**Limit of detection** calculated as the concentration of IL-6 (pig) corresponding to the NSB average plus three standard deviation is ≤0.08 ng/mL.
Troubleshooting

Absorbance values are too low:
- one reagent has not been dispensed,
- incorrect preparation,
- assay performed before reagents reached room temperature,
- reading time not long enough.

High signal and background in all wells:
- inefficient washing,
- overdeveloping (incubation time should be reduced),
- high ambient temperature.

High dispersion of duplicates:
- poor pipetting technique or irregular plate washing.

These are a few examples of troubleshooting that may occur. If you need further explanation, Bertin Pharma will be happy to assist you. Feel free to contact our technical support staff by phone (+33 (0)139 306 036), fax (+33 (0)139 306 299) or E-mail (bioreagent@bertinpharma.com), and be sure to indicate the batch number of the kit (see outside the box).

Bertin Pharma proposes EIA Training kit #B05005 and EIA workshop upon request. For further information, please contact our Marketing Department by phone (+33 (0)139 306 260) or E-mail (marketing@bertinpharma.com).

Bibliography

1. Toshio T., Masashi N., Tadamitsu K. IL-6 in Inflammation, Immunity, and Disease. Cold Spring Harb Perspect Biol. 2014 Sep 4;6(10)


Bertin Pharma, over the last decades, has been developing and marketing over 100 biomarker assays, pre-analytical products, kits, antibodies and biochemicals thanks to its innovative work in research and development. Our core areas are orientated to inflammation, oxidative injury, endocrinology, diabetes, obesity, hypertension, neurodegenerative diseases, HIV, prion diseases, pharmacokinetics and metabolism.

Bertin Pharma is active worldwide either with direct sales or through our qualified and trained international distribution network from the United States to Japan.

We are able to provide you with local technical support to use at ease our products.

For further information, please send your request to bioreagent@bertinpharma.com

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