IL6 Chemi-Luminescent ELISA Kit (Dog) (OKCD03684)

Lot# KD0938

Instructions for use

For the quantitative measurement of IL6 in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants and other biological fluids.

Variation between lots can occur. Refer to the manual provided with the kit.

This product is intended for research use only.

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1. Background

Principle

Aviva Systems Biology IL6 Chemi-Luminescent ELISA Kit (Dog) (OKCD03684) is based on standard sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific for IL6 has been pre-coated onto a 96-wellplate (12 x 8 Well Strips). Standards or test samples are added to the wells, incubated and removed. A biotinylated detector antibody specific for IL6 is added, incubated and followed by washing. Avidin-Peroxidase Conjugate is then added, incubated and unbound conjugate is washed away. An enzymatic reaction is produced through the addition of a luminol substrate which is catalyzed by the HRP to produce light emission. The light emission is read by a luminometer (or photo-multiplier equipped instrument) and the intensity of the emitted light is proportional to the amount of sample IL6 captured in well.

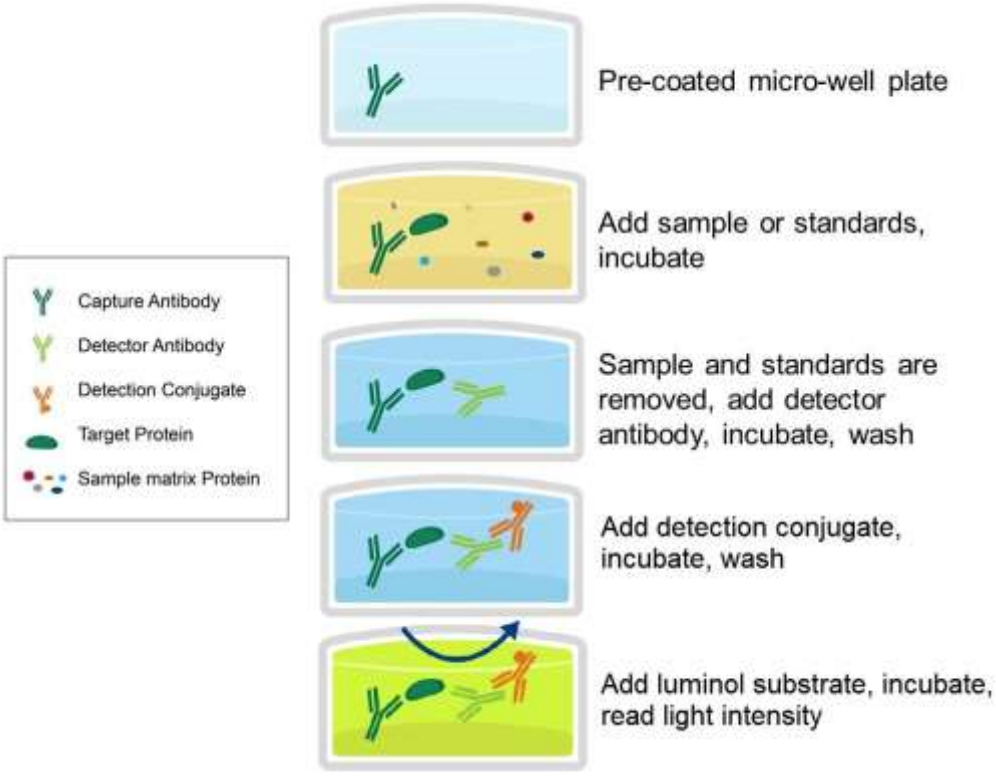
Background

Cytokine with a wide variety of biological functions. It is a potent inducer of the acute phase response. Plays an essential role in the final differentiation of B-cells into Ig-secreting cells Involved in lymphocyte and monocyte differentiation. Acts on B-cells, T-cells, hepatocytes, hematopoietic progenitor cells and cells of the CNS. Required for the generation of T(H)17 cells. Also acts as a myokine. It is discharged into the bloodstream after muscle contraction and acts to increase the breakdown of fats and to improve insulin resistance. It induces myeloma and plasmacytoma growth and induces nerve cells differentiation .

General Specifications

General Specifications	
Range	1.37 - 1,000 pg/mL
LOD	< 0.49 pg/mL (Derived by linear regression of OD_{450} of the Mean Blank + 2xSD)
Specificity	Dog IL6 UniProt ID: P41323 Gene ID: 403985 Target Alias: Interleukin-6; IL-6
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins

2. Assay Summary



3. Storage and Stability

• Open kit immediately upon receipt. Store components at -20°C (NOTE: exceptions below) for 6 months or until expiration date. Avoid any freeze/thaw cycles.

4. Kit Components

•The following reagents are the provided contents of the kit.

Description	Quantity	Storage Conditions
Anti-IL6 Microplate	96 Wells (12 x 8 Well strips)	-20°C for 6 months
IL6 Lyophilized Standard	2 x 500 pg	
100X Biotinylated IL6 Detector Antibody	1 x 120 µL	
100X Avidin-HRP Conjugate	1 x 120 µL	
Standard Diluent	1 x 20 mL	4°C for 6 months
Detector Antibody Diluent	1 x 12 mL	
Conjugate Diluent	1 x 12 mL	
30X Wash Buffer	1 x 20 mL	
Substrate Diluent	1 x 10 mL	
100X Luminol Substrate	1 × 2 mL	

5. Precautions

• Read instructions fully prior to beginning use of the assay kit.

• Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.

• Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.

• For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).

• Kit cannot be used beyond the expiration date on the label.

6. Required Materials Not Supplied

• Luminometer or photo-multiplier tube (PMT) equipped microplate reader capable of the following parameters: lag time 30.0 seconds, read time 1.0 seconds per well.

• Automated plate washer (optional).

• Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.

• Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.

• New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.

• Absorbent paper or paper toweling.

• Distilled or deionized ultrapure water.

• 37°C Incubator (optional)

7. Technical Application Tips

• Do not mix or substitute components from other kits.

• To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.

• Samples exhibiting light intensity measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.

• Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.

• Replicate wells are recommended for standards and samples.

• Cover microplate while incubating to prevent evaporation.

• Do not allow the microplate wells dry at any point during the assay procedure.

• Do not reuse tips or tube to prevent cross contamination.

• Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.

• Completely remove of all liquids when washing to prevent cross contamination.

• Prepare reagents immediately prior to use and do not store, with the exception of the top standard.

• Equilibrate all materials to ambient room temperature prior to use (standards exception).

• For optimal results for inter- and intra-assay consistency, equilibrate all materials to room temperature prior to performing assay (standards exception) and perform all incubations at 37°C.

• Pipetting less than 1 µL is not recommended for optimal assay accuracy.

• Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.

• Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.

• Samples containing precipitates, fibrin strands or bilirubin, or are hemolytic or lipemic might cause inaccurate results due to interfering factors.

• Luminol Substrate is easily contaminated and labile. Handle carefully and protect from light.

8. Reagent Preparation

• Equilibrate all materials to room temperature prior to use and use immediately.

8.1 Dog IL6 Assay Standards

8.1.1 Prepare the IL6 standards no greater than 2 hours prior to performing experiment. Standards should be held on ice until use in the experiment.

8.1.2 Reconstitute one vial of the provided **500 pg Lyophilized IL6 Standard** for each experiment. Prepare a stock **1,000 pg/mL Standard** by reconstituting one tube of **Lyophilized IL6 Standard** as follows:

8.1.2.1 Gently spin or tap the vial at 6,000 – 10,000 rpm for 30 seconds to collect all material at the bottom.

8.1.2.2 Add **500 µL** of **Standard Diluent** to the vial.

8.1.2.3 Seal the vial then mix gently and thoroughly.

8.1.2.4 Leave the vial at ambient temperature for 15 minutes.

8.1.3 Prepare a set of serially diluted standards as follows:

8.1.3.1 Label tubes with numbers 2 – 8.

8.1.3.2 Use the reconstituted 1,000 pg/mL **IL6 Standard** from step 8.1.2 as the high standard point (Tube #1).

8.1.3.3 Add **600 µL** of **Standard Diluent** to Tube #'s 2 – 8.

8.1.3.4 Prepare **Standard #2** by adding 300 µL of 1,000 pg/mL **IL6** (Tube #1) to Tube #2. Mix gently and thoroughly.

8.1.3.5 Prepare **Standard #3** by adding 300 µL of **Standard #2** from Tube #2 to Tube #3. Mix gently and thoroughly.

8.1.3.6 Prepare further serial dilutions through Tube #7. Reference the table below as a guide for serial dilution scheme.

8.1.3.7 Tube #8 is a blank standard (only **Standard Diluent**), which should be included with every experiment.

Standard Number (Tube)	Standard To Dilute	Volume Standard to Dilute (µL)	Volume Standard Diluent (µL)	Total Volume (µL)	Final Concentration
1	500 pg/mL Reconstituted Standard	NA	500	500	1,000 pg/mL
2	1,000 pg/mL	300	600	900	333.33 pg/mL
3	333.33 pg/mL	300	600	900	111.11 pg/mL
4	111.11 pg/mL	300	600	900	37.04 pg/mL
5	37.04 pg/mL	300	600	900	12.35 pg/mL
6	12.35 pg/mL	300	600	900	4.12 pg/mL
7	4.12 pg/mL	300	600	900	1.37 pg/mL
8	NA	0	600	600	0.0 (Blank)

8.2 1X Biotinylated IL6 Detector Antibody

8.2.1 Prepare the **1X Biotinylated IL6 Detector Antibody** immediately prior to use by diluting the **100X Biotinylated IL6 Detector Antibody** 1:100 with **Detector Antibody Diluent**.

8.2.2 For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of **100X Biotinylated IL6 Detector Antibody** to 990 µL **Detector Antibody Diluent**.

8.2.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

8.3 1X HRP-Avidin Conjugate

8.3.1 Prepare the **1X Avidin-HRP Conjugate** immediately prior to use by diluting the **100X Avidin-HRP Conjugate** 1:100 with **Conjugate Diluent**.

8.3.2 For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of **100X Avidin-HRP Conjugate** to 990 µL **Conjugate Diluent**.

8.3.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

8.4 1X Luminol Substrate

8.4.1 Prepare the **1X Luminol Substrate** immediately prior to use by diluting the **100X Luminol Substrate** 1:100 with **Substrate Diluent**.

8.4.2 For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of **100X Luminol Substrate** to 990 µL **Substrate Diluent**.

8.4.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

8.5 1X Wash Buffer

8.5.1 If crystals have formed in the 30X **Wash Buffer** concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.

8.5.2 Add the entire 20 mL contents of the 30X **Wash Buffer** bottle to 580 mL of ultra-pure water to a clean > 1,000 mL bottle or other vessel.

8.5.3 Seal and mix gently by inversion. Avoid foaming or bubbles.

8.5.4 Store the **1X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 week. Do not freeze.

8.6 Microplate Preparation

- Micro-plates are provided ready to use and do not require rinsing or blocking.
- Unused well strips should be returned to the original packaging, sealed and stored at 4°C.
- Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

9. Sample Preparation

9.1 Sample Preparation and Storage

• Store samples to be assayed at 2-8°C for 24 hours prior being assayed.

• For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

• Samples not indicated in the manual must be tested to determine if the kit is valid.

• Prepare samples as follows:

• **Serum** - Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at 1,000 x g. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

• **Plasma** - Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

• **Tissue Homogenates** – Rinse 100 mg tissue with 1X PBS then homogenize in 1 mL of 1X PBS and store overnight at -20°C. Perform two freeze-thaw cycles to break the cell membranes then centrifuge for 5 minutes at 5,000 x g, 2-8°C. Remove the supernatant and assay immediately. Alternatively, aliquot and store samples at -20°C or -80°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

• **Cell Lysates** – Cells need to be lysed before assaying according to the following directions:
1. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1,000×g for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells three times in cold PBS.
3. Resuspend cells in fresh lysis buffer with concentration of 10^7 cells/ml. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified.
4. Centrifuge at 1,500×g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or aliquot and store at ≤ -20°C

• **Cell culture supernatants and Other biological fluids** - Centrifuge samples for 20 minutes at 1000 × g. Remove particulates and assay immediately or store samples in aliquot at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

10. Assay Procedure

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

• Optimal results for intra- and inter-assay reproducibility will be obtained when performing incubation steps at 37°C as indicated below.

10.1 Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.

10.2 Add 100 µL of serially titrated standards, diluted samples or blank into wells of the **Anti-IL6 Microplate**. At least two replicates of each standard, sample or blank is recommended.

10.3 Cover the plate with the plate sealer and incubate at 37°C for 60 minutes.

10.4 Remove the plate sealer and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.5 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.6 Add 100 µL of prepared **1X Biotinylated IL6 Detector Antibody** to each well.

10.7 Cover with the plate sealer and incubate at 37°C for 60 minutes.

10.8 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.9 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.10 Wash plate 3 times with **1X Wash Buffer** as follows:

10.10.1 Add 300 µL of **1X Wash Buffer** to each assay well.

10.10.2 Incubate for 1-2 minutes.

10.10.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.10.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.10.5 Repeat steps 10.10.1 through 10.10.4 **two** more times.

10.11 Add 100 µL of prepared **1XAvidin-HRP Conjugate** into each well, cover with plate sealer and incubate at 37°C for 30 minutes.

10.12 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.13 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.14 Wash plate **5 times** with **1X Wash Buffer** as in Step 10.10.

10.15 Add 100 µL of **1X Luminol Substrate** to each well, cover with plate sealer and incubate at 37°C for 10 minutes **in the dark**.

10.16 Read the RLU (relative light units) with a standard luminometer or photo-multiplier tube equipped instrument.

11. Calculation of Results

For analysis of the assay results, calculate the **Relative Light Units (RLU)** for each test or standard well as follows

(Relative Light Units) = (Mean Sample Well Light Unit Emission) – (Mean Blank Well Light Unit Emission)

The standard curve is generated by plotting the mean replicate **RLU** of each standard serial dilution point vs. the respective standard concentration. The **IL6** concentration contained in the samples can be interpolated by using linear regression of each mean sample **RLU** against the standard curve. This is best achieved using curve fitting software.

Note: If the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

12. Typical Expected Data

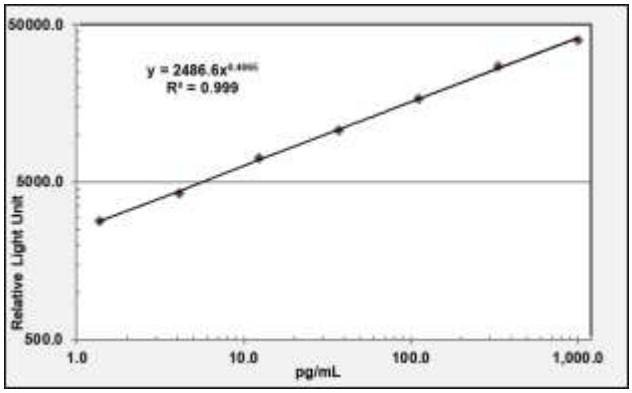
12.1 Reproducibility

Intra-assay Precision: 3 samples with known low, middle and high levels IL6 were tested with 20 replicates on one plate, respectively. Inter-assay Precision: 3 samples with known low, middle and high level IL6 were tested on 3 different plates, 8 replicates in each plate.

Sample	Intra-Assay			Inter-Assay		
	1	2	3	1	2	3
Sample	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (pg/ml)	5.71	35.46	315.24	6.01	41.34	314.23
SD	0.411	2.269	16.708	0.439	2.770	16.340
CV (%)	7.2	6.4	5.3	7.3	6.7	5.2

12.2 Typical standard curve

This standard curve is for demonstration purposes only. An assay specific standard curve should be performed with each assay.



pg/mL	Relative Light Units		Mean RLU	Blank Subtracted
	Rep 1	Rep 2		
1,000	39782	40836	40309	39729
333.3	27287	26565	26926	26346
111.1	17032	17154	17093	16513
37.04	10698	10772	10735	10155
12.35	7156	7254	7205	6625
4.12	4247	4133	4190	3610
1.37	2842	2854	2848	2268
Blank	574	586	580	NA

12.3 Linearity

Kit linearity evaluated by replicate testing (n=4) serially diluted serum spiked with known concentration of IL6. Results are expressed as the percentage of the expected concentration measurement.

Sample	1:2	1:4	1:8	1:16
Serum (n=5)	85-99%	79-94%	78-103%	94-101%
EDTA Plasma (n=5)	82-97%	83-98%	93-107%	80-96%
heparin Plasma (n=5)	78-93%	87-96%	80-92%	86-104%

12.4 Recovery

The following matrices were spiked with known concentration of IL6. Recovery is expressed as the percentage of the expected concentration measurement.

Matrix	Recovery Range (%)	Average (%)
Serum (n=5)	78-93	86
EDTA plasma (n=5)	92-105	99
Heparin Plasma (n=5)	80-97	92

13. Technical Resources

Technical Support:

For optimal service please be prepared to supply the lot number of the kit used.

USA

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