IL10 ELISA Kit (Mouse) (OKBB00194)

Lot# KD1118

Instructions for Use

For the quantitative measurement of IL10 in cell culture supernatants, serum.

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 IL10 ELISA Kit (Mouse) (OKBB00194) is based on standard sandwich enzyme-linked immune-sorbent assay technology. An antibody specific for IL10 has been pre-coated onto 96-wellplate (12 x 8 Well Strips). Standards (E.coli, S19-S178) and test samples are added to the wells, incubated and removed. A biotinylated detector antibody specific for IL10 is added, incubated and followed by washing. Avidin-Biotin-Peroxidase Complex is then added, incubated and unbound complex is washed away. An enzymatic reaction is visualized through the addition of TMB substrate which is catalyzed by HRP to produce a blue color product that changes to yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm and is quantitatively proportional to the amount of sample Mouse IL10 captured in the well.

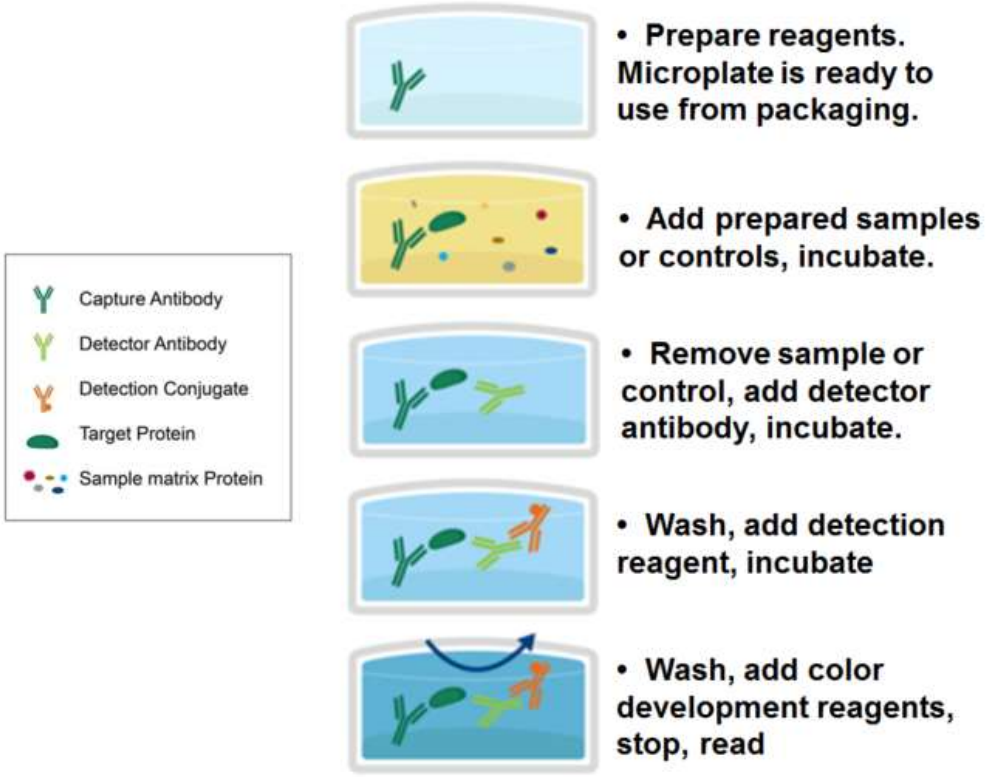
Background

Interleukin-10, also called cytokine synthesis inhibitory factor, is implicated in tumorigenesis, and it has been shown that polymorphisms in its gene promoter correlate with differential amounts of production. IL-10 is an important cytokine with anti-inflammatory, anti-immune, and antifibrotic functions. It is also an important regulatory cytokine whose involvement extends into diverse areas of the Mouse immune system. IL-10 is a recently described natural endogenous immunosuppressive cytokine that has been identified in Mouse, murine, and other organisms. IL-10 significantly affects chemokine biology, because Mouse IL-10 inhibits chemokine production and is a specific chemotactic factor for CD8+ T cells. It suppresses the ability of CD4+ T cells, but not CD8+ T cells, to migrate in response to IL-8. Interleukin-10 gene polymorphisms and interleukin-10 production capability may contribute to the development of skin squamous cell carcinomas after renal transplantation. The interleukin-10 locus contributes to the heritability of psoriasis susceptibility. With regard to sudden infant death, IL-10 is of special interest. This is an immunoregulatory cytokine that plays an important role in the development of infectious disease. The mIL-10 gene is mapped to mouse chromosome 1 and the hIL-10 gene is also mapped to Mouse chromosome 1. The Standard product used in this kit is recombinant mouse IL-10 , consisting of 160 amino acid with the molecular mass of 18KDa.

General Specifications

General Specifications	
Range	15.6 - 1,000 pg/mL
Sensitivity	< 1 pg/mL (Derived by linear regression of OD_{450} of the Mean Blank + 2xSD)
Specificity	Mouse IL10 UniProt ID: P18893 Gene ID: 16153 Target Alias: Interleukin-10; Cytokine synthesis inhibitory factor; CSIF; IL-10
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins

2. Assay Summary



3. Storage and Stability

• Upon receipt store kit at 4°C until the expiration date. Avoid multiple freeze/thaw cycles.

4. Kit Components

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

Description	Quantity	Storage Conditions
96-Well Plate Pre-coated with Anti-Mouse IL-10 Antibody	96 Wells (12 x 8 Well Strips)	Store at 4°C until expiration date
Lyophilized Recombinant Mouse IL-10 Standard	2 x 10 ng	
100X Biotinylated Anti-Mouse IL-10 Antibody	1 x 130 µL	
100X Avidin-Biotin-Peroxidase Complex (ABC)	1 x 130 µL	
Sample Diluent Buffer	1 x 30 mL	
Antibody Diluent Buffer	1 x 12 mL	
ABC Diluent Buffer	1 x 12 mL	
TMB Color Developing Agent	1 x 10 mL	
TMB Stop Solution	1 x 10 mL	
10X Wash Buffer	1 x 30 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).

6. Required Materials Not Supplied

• Microplate reader capable of reading absorbance at 450 nm.

• 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.

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 OD measurements higher than the highest Standard should be diluted further in the appropriate sample dilution buffers.

• Inspect all reagents prior to use. Components should contain no particulates or cloudiness and should be colorless.

• Prior to using the kit, briefly spin component tubes to collect all reagent 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 in inter- 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 or fibrin strands or which are hemolytic of lipemic might cause inaccurate results due to interfering factors.

8. Reagent Preparation

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

8.1 IL10 Assay Standards

8.1.1 Prepare the IL10 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 of the provided 10 ng **Lyophilized IL10 Standard**. Use one for each experiment. Prepare a stock 10,000 pg/mL **Standard** by reconstituting one tube of **Lyophilized IL10 Standard** as follows:

8.1.2.1 Gently spin or tap the vial to collect all material at the bottom.

8.1.2.2 Add 1 mL of **Sample Diluent Buffer** to the vial.

8.1.2.3 Seal then mix gently and thoroughly.

8.1.2.4 Leave the vial to sit at ambient temperature for 10 minutes.

8.1.3 Prepare a set of seven serially diluted Standards as follows:

8.1.3.1 Label tubes with numbers 1 – 8.

8.1.3.2 Add 300 µL of **Sample Diluent Buffer** to Tube **#**'s 1 – 7.

8.1.3.3 Prepare a **1,000 pg/mL Standard #1** in by adding 100 µL of the **10,000 pg/mL** reconstituted **IL10 Standard** to 900 µL of **Sample Diluent Buffer** in Tube#1. Mix gently and thoroughly.

8.1.3.4 Prepare **Standard #2** by adding 300 µL of **Standard#1** from 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 **Sample Diluent Buffer**), which should be included with every experiment.

Standard Number (Tube)	Sample To Dilute	Volume Standard (µL)	Volume Sample Diluent Buffer (µL)	Total Volume (µL)	Final Concentration
1	10,000 pg/mL IL-10 Standard	100	900	1,000	1,000 pg/mL
2	1,000 pg/mL	300	300	600	500 pg/mL
3	500 pg/mL	300	300	600	250 pg/mL
4	250 pg/mL	300	300	600	125 pg/mL
5	125 pg/mL	300	300	600	62.5 pg/mL
6	62.5 pg/mL	300	300	600	31.2 pg/mL
7	31.2 pg/mL	300	300	600	15.6 pg/mL
8	NA	0	300	300	0.0 (Blank)

8.2 1X Biotinylated Anti-Mouse IL10 Antibody

8.2.1 Prepare the **1X Biotinylated Anti-Mouse IL10 Antibody immediately** prior to use by diluting the **100X Biotinylated Anti-Mouse IL10 Antibody** 1:100 with Antibody Diluent Buffer.

8.2.2 For each 8-well strip to be used in the experiment prepare 1,000 µL by adding 10 µL of **100X Biotinylated Anti-Mouse IL10 Antibody to** 990 µL **Antibody Diluent Buffer**.

8.2.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure.

8.3 1X Avidin-Biotin-Peroxidase Complex (ABC)

8.3.1 Prepare the **1X Avidin-Biotin-Peroxidase Complex (ABC)** immediately prior to use by diluting the **100X Avidin-Biotin-Peroxidase Complex (ABC) 1:100** with **ABC Dilution Buffer**.

8.3.2 For each 8-well Strip to be used in the experiment prepare 1,000 µL by adding 10 µL of **100X Avidin-Biotin-Peroxidase Complex (ABC)** to 990 µL **ABC Dilution Buffer**.

8.3.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure.

8.4 1X Wash Buffer

8.4.1 Add 270 mL of ultra-pure water to a clean > 500 mL bottle or other vessel.

8.4.2 Add the entire 30 mL contents of the **10X Wash Buffer** bottle to the water.

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

8.4.4 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.

8.5 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.

• Clear samples by centrifugation as follows:

- **Cell culture supernatants** - Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.
- **Serum** - Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.

9.2 Sample Dilution

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range.

• Prepare 150 µL sample for each replicate to be assayed.

• Dilute samples with **Sample Diluent Buffer**.

• Mix diluted samples gently and thoroughly.

• Pipetting less than 2 µL is not recommended for optimal assay accuracy.

• Refer to the following table for recommended sample dilution guidelines based on the dynamic range of this kit:

Estimated Sample Target Concentration		Dilution Level	Sample Volume For Two Replicates	Sample Diluent Buffer For Two Replicates
High Concentration	10-100 ng/mL	1:100	1 µL	99 µL
Medium Concentration	1-10 ng/mL	1:10	10 µL	90 µL
Low Concentration	15.6-1,000 pg/mL	1:2	50 µL	50 µL
Very Low Concentration	≤ 15.6 pg/mL	1:2 or No Dilution	-	-

10. Assay Procedure

• The ABC Working Solution, TMB Color Developing Agent and TMB Stop Solution must be kept warm at 37°C for 30 minutes prior to use. Equilibrate all other reagents and materials to ambient room temperature prior to use in the procedure.

• To control for small potential variations in micro well-plate and day to day ambient temperature fluctuations, equilibrate all reagents prior to use and perform all incubation steps at to 37°C for optimal consistency and reproducibility.

10.1 Add 100 µL of serially titrated standards, diluted samples or blank into wells of the pre-coated well plate. At least two replicates of each standard, sample or blank is recommended.

10.2 Cover the plate with the plate sealer and incubate at 37°C for 90 minutes.

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

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.

10.5 Add 100 µL of prepared **1X Biotinylated Anti-Mouse IL10 Antibody to each well.**

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

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

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

10.7.2 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.

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

10.7.4 Incubate for 1 minute.

10.7.5 Repeat steps 10.7.1 through 10.7.4 **two** more times.

10.8 Add 100 µL of prepared **1X Avidin-Biotin-Peroxidase Complex (ABC) into** each well, cover with plate sealer and incubate at 37°C for 30 minutes.

10.9 Wash plate 5 times with **1X Wash Buffer as** follows:

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

10.9.2 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.

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

10.9.4 Incubate for 1 minute.

10.9.5 Repeat steps 10.9.1 through 10.9.4 **four** more times.

10.10 Add 90 µL of **TMB Color Developing Agent** to each well, cover with plate sealer and incubate at 37°C **in the dark** for 25-30 minutes.

(NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.)

10.11 Add 100 µL of **TMB Stop Solution** to each well. Well color should change to yellow immediately.

10.12 Read the O.D. absorbance at 450 nm with a standard microplate reader within 30 minutes of stopping the reaction in step 10.11.

11. Calculation of Results

For analysis of the assay results, calculate the **Relative OD_{450}** for each test or Standard well as follows:

$$(\text{Relative } OD_{450}) = (\text{Well } OD_{450}) - (\text{Mean Blank Well } OD_{450})$$

The Standard curve is generated by plotting the mean replicate Relative **OD_{450}** of each Standard serial dilution point vs. the respective Standard concentration. The **IL10** concentration contained in the samples can be interpolated by using linear regression of each mean sample **Relative OD_{450}** against the Standard curve. This is best achieved using curve fitting software.

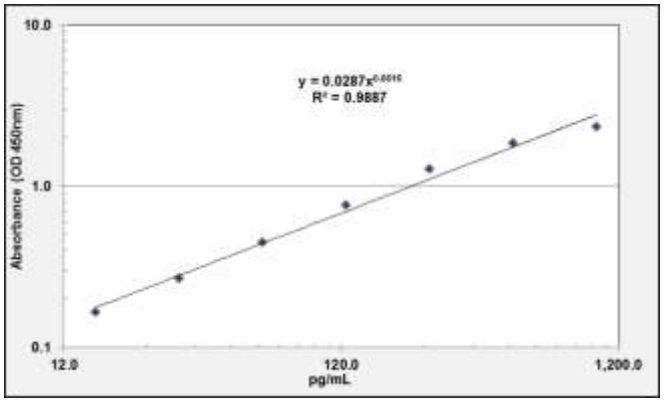
Note: if the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

12. Typical Expected Data

12.1 Typical absorbance values. Expected absorbance for Standards when TMB incubation is performed for 20 minutes at 37°C and measured at OD_{450}.

Standard Number	8	7	6	5	4	3	2	1
Standard Concentration (pg/mL)	0	15.6	31.2	62.5	125	250	500	1,000
OD_{450}	0.075	0.165	0.267	0.450	0.758	1.278	1.840	2.325

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



12.3 Reproducibility

	Intra-Assay			Inter-Assay		
Sample ID	1	2	3	1	2	3
n =	16	16	16	24	24	24
Mean Measured Concentration (pg/mL)	33	219	466	35	203	420
Standard Deviation	1.88	14.01	19.1	2.66	16.64	25.7
Consistency (%CV)	5.7	6.4	4.1	7.6	8.2	6

13. Technical Resources

13.1 References

13.1.1 Alamartine, E.; Berthoux, P.; Mariat, C.; Cambazard, F.; Berthoux, F. Interleukin-10 promoter polymorphisms and susceptibility to skin squamous cell carcinoma after renal transplantation. J. Invest. Derm. 120: 99-103, 2003.

13.1.2 Grove, J.; Daly, A. K.; Bassendine, M. F.; Gilvarry, E.; Day, C. P. Interleukin 10 promoter region polymorphisms and susceptibility to advanced alcoholic liver disease. Gut 46: 540-545, 2000.

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13.1.5 Asadullah, K.; Eskdale, J.; Wiese, A.; Gallagher, G.; Friedrich, M.; Sterry, W. Interleukin-10 promoter polymorphism in psoriasis. J. Invest. Derm. 116: 975-978, 2001.

13.1.6 Opdal, S. H.; Opstad, A.; Vege, A.; Rognum, T. O. IL10 gene polymorphisms are associated with infectious cause of sudden infant death. Hum. Immun. 64: 1183-1189, 2003.

13.1.7 Kim, J. M.; Brannan, C. I.; Copeland, N. G.; Jenkins, N. A.; Khan. Structure of the mouse IL-10 gene and chromosomal localization of the mouse and Mouse genes. J. Immun. 148: 3618-3623, 1992.

13.2 Technical Support

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