Version 30-11/24

User's Manual

Porcine IL-10 ELISA Kit

REF **DEIA2817**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

For the quantitative determination of porcine Interleukin 10 (IL-10) concentrations in cell culture supernates, serum, and EDTA plasma.

General Description

Interleukin 10, also known as cytokine synthesis inhibitory factor (CSIF), is the charter member of the IL-10 cytokine family. This family currently comprises IL-10, IL-19, IL-20, IL-22, IL-24, and IL-26/AK155. All IL-10 family members are secreted α-helical proteins. Porcine IL-10 is a secreted, possibly glycosylated, polypeptide with an 18 kDa molecular weight. Based on human studies, porcine IL-10 is likely to circulate as a nondisulffde-linked homodimer. Porcine IL-10 is synthesized as a 175 amino acid (aa) precursor with an 18 aa signal sequence and a 157 aa mature form. The mature segment has one potential N-linked glycosylation site plus four cysteines which form two intrachain disulffde bridges. Mature porcine IL-10 shows 71%, 70%, 76%, 75%, 77%, and 71% aa sequence identity to rat, mouse, human, guinea pig, canine, and cotton rat IL-10, respectively. Upon activation, mammalian cells known to secrete IL-10 include NK cells, cytotoxic CD8+ T cells secreting Th2-like cytokines, CD4+CD45RA- (memory) Th1 and Th2 cells, macrophages, monocytes, CD5+ and CD5-B cells, dendritic cells, hepatic stellate (Ito cells), keratinocytes, melanoma cells, mast cells, placental cytotrophoblasts, and fetal erythroblasts.

The functional receptor for IL-10 (IL-10 R) in pigs has not been reported. By analogy to human, it would be expected to be composed of two 110 kDa α-chains (or IL-10 R1) and two 75 kDa β-chains (or IL-10 R2). The α-chain binds IL-10 and transduces a signal in the presence of a β-chain complex. Both receptors are members of the class II cytokine receptor family (CRF2) that is characterized by the presence of type III ffbronectin domains and conserved tryptophans. This class does not possess the WSXWS motif characteristic of the class I CRF. There is no signiffcant aa sequence identity (< 30%) between human IL-10 R1 and IL-10 R2. IL-10 has myriad effects on a variety of cell types. On activated B cells, IL-10 can induce plasma cell formation and the secretion of either IgG or IgA (in the presence of TGF-β1 and/or IL-4). In the presence of IL-2, CD56+ NK cells will respond to IL-10 with increased proliferation plus IFN-γ and TNF-α secretion. Conversely, on macrophages, IL-10 is known to downregulate IL-1, TNF-α, and IL-6 production. On dendritic cells, IL-10 has been shown to interfere with antigen-presenting cell function by downmodulating stimulatory and co-stimulatory molecules. On monocytes, IL-10 is reported to direct monocyte differentiation into cytotoxic CD16+ macrophages rather than antigen-presenting dendritic cells.

The Porcine IL-10 Immunoassay is a 4.5 hour solid phase ELISA designed to measure porcine IL-10 levels in cell culture supernates, serum, and EDTA plasma. It contains E. coli-expressed recombinant porcine IL-10 and antibodies raised against the recombinant protein. Results obtained for naturally occurring porcine IL-10 showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that this kit can be used to determine relative mass values of natural porcine IL-10.

Principles of Testing

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody speciffc for porcine IL-10 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-10 present is bound by the immobilized antibody. After washing away any

unbound substances, an enzyme-linked polyclonal antibody speciffc for porcine IL-10 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of IL-10 bound in the initial step. The sample values are then read from the standard curve.

Reagents And Materials Provided

1. Porcine IL-10 Microplate, 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody speciffc for porcine IL-10. Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8°C.** Provided this is within the expiration date of the kit.

2. Porcine IL-10 Standard, 2 vials of recombinant porcine IL-10 in a buffered protein base with preservatives; lyophilized. Refer to the vial label for reconstitution volume. Use a new standard and control for each assay. Discard after use.

3. Porcine IL-10 Control, 2 vials of recombinant porcine IL-10 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range speciffed on the label. Use a new standard and control for each assay. Discard after use.

4. Porcine IL-10 Conjugate, 23 mL of a polyclonal antibody speciffc for porcine IL-10 conjugated to horseradish peroxidase with preservatives.May be stored for up to 1 month at 2-8°C.*

5. Assay Diluent CD1W, 12 mL of a buffered protein base with preservatives. May be stored for up to 1 month at 2-8 °C.*

6. Calibrator Diluent CD6-33, 21 mL of a buffered protein base with preservatives. May be stored for up to 1 month at 2-8 °C.*

7. Wash Buffer Concentrate, 21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. May turn yellow over time. May be stored for up to 1 month at 2-8°C.*

8. Color Reagent A, 12 mL of stabilized hydrogen peroxide. May be stored for up to 1 month at 2-8°C.*

9. Color Reagent B, 12 mL of stabilized chromogen (tetramethylbenzidine). May be stored for up to 1 month at 2-8°C.*

10. Stop Solution, 23 mL of diluted hydrochloric acid. May be stored for up to 1 month at 2-8 °C.*

11. Plate Sealers, 4 adhesive strips.

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.
6. Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.

7. Test tubes for dilution of standards and samples.

Storage

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

Specimen Collection And Preparation

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

1. Cell Culture Supernates: Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

2. Serum: Allow blood samples to clot for 2 hours at room temperature before centrifuging for 30 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

3. Plasma: Collect plasma using EDTA as an anticoagulant. Centrifuge for 30 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: Heparin and citrate plasma have not been validated for use in this assay.

SAMPLE PREPARATION

Cell culture supernate, serum, and EDTA plasma samples require a 2-fold dilution prior to assay.

A suggested 2-fold dilution is 120 µL of sample + 120 µL of Calibrator Diluent CD6-33.

Reagent Preparation

Bring all reagents to room temperature before use.

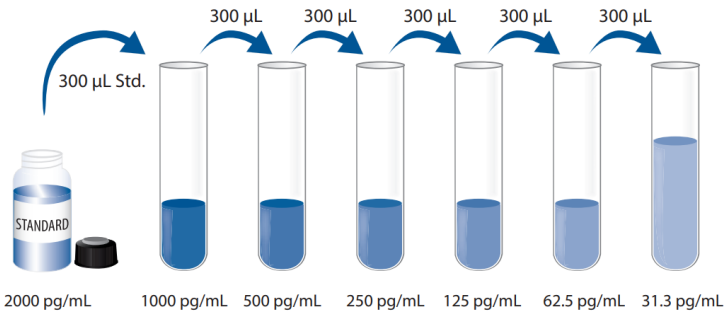
1. Porcine IL-10 Control. Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

2. Wash Buffer. If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buff er Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

3. Substrate Solution. Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 120 µL of the resultant mixture is required per well.

4. Porcine IL-10 Standard. Refer to the vial label for reconstitution volume. Reconstitute the Porcine IL-10 Standard with Calibrator Diluent CD6-33. Do not substitute other diluents. This reconstitution produces a stock solution of 2000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 300 µL of Calibrator Diluent CD6-33 into each tube. Use the standard stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Porcine IL-10 Standard (2000 pg/mL) serves as the high standard. Calibrator Diluent CD6-33 serves as the zero standard (0 pg/mL).



Assay Procedure

Bring all reagents and samples to room temperature before use. It is recommended that all standards, control, and samples be assayed in duplicate.

1. Prepare reagents, standard dilutions, control, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1W to each well.
4. Add 100 µL of standard, control or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Porcine IL-10 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 120 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature on the benchtop. Protect from light.
9. Add 120 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

    ***Samples require dilution. See Sample Preparation section.**

Calculation

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-

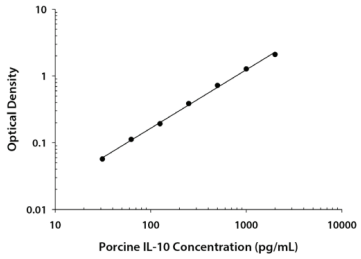
axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the porcine IL-10 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

Since samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

This immunoassay is calibrated against a highly purified E. coli-expressed recombinant porcine IL-10 produced.

Typical Standard Curve

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.068 0.074	0.071	—
31.3	0.127 0.128	0.128	0.057
62.5	0.179 0.187	0.183	0.112
125	0.262 0.263	0.263	0.192
250	0.449 0.465	0.457	0.386
500	0.788 0.798	0.793	0.722
1000	1.335 1.359	1.347	1.276
2000	2.167 2.179	2.173	2.102

Evaluation

Serum/Plasma: Fifteen samples were evaluated for detectable levels of porcine IL-10 in this assay. One EDTA plasma sample measured 118 pg/mL, while all remaining samples measured below the lowest standard, 31.3 pg/mL.

Cell Culture Supernates: Porcine peripheral blood mononuclear cells (5×10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum and stimulated with 50 ng/mL PMA and 500 ng/mL calcium ionomycin for 20 hours. An aliquot of the cell culture supernate was removed, assayed for porcine IL-10, and measured 254 pg/mL.

Precision

Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of kit components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	88	264	791	89	264	802
Standard deviation	3.7	6.8	27.5	6.4	11.8	54.7
CV (%)	4.2	2.6	3.5	7.2	4.5	6.8

Sensitivity

Fourteen assays were evaluated and the minimum detectable dose (MDD) of porcine IL-10 ranged from 1.8-5.5 pg/mL. The mean MDD was 3.5 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity

This assay recognizes natural and recombinant porcine IL-10.

Linearity

To assess the linearity of the assay, samples spiked with high concentrations of porcine IL-10 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay as directed in the Sample Preparation section.

		Cell culture supernates (n=4)	Serum (n=4)	EDTA plasma (n=4)
1:2	Average % of Expected	105	103	107
	Range (%)	103-106	100-106	104-109
1:4	Average % of Expected	99	99	102
	Range (%)	97-100	94-104	98-105
1:8	Average % of Expected	92	93	94
	Range (%)	90-94	85-98	86-98
1:16	Average % of Expected	87	91	92
	Range (%)	84-90	80-100	85-96

Recovery

The recovery of porcine IL-10 spiked to levels throughout the range of the assay in various matrices was evaluated. Samples were diluted prior to assay as directed in the Sample Preparation section.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=4)	106	101-109%
Serum (n=4)	94	84-102%
EDTA plasma (n=4)	92	80-100%

Precautions

Calibrator Diluent CD6-33 contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction.

Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.