REFERENCES

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2.Rau FC, et al. (2009) B7-1/2 (CD80/CD86) direct signaling to B cells enhances IgG secretion. J Immunol. 183(12): 7661-71.

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Rat CD86 Immunoassay

Catalog Number:SEKR-0083

For the quantitative determination of Rat CD86 concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

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LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of CD86 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

The linearity of the assay

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	91	102
	Range(%)	83-101	95-108
1:4	Average% of Expected	96	105
	Range(%)	87-109	97-116

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 9.3 pg/mL.
SPECIFICITY: This assay recognizes both natural and recombinant Rat CD86. The factors listed below were prepared at 50ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.
RECOVERY: The recovery of CD86 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of CD86 in two matrices

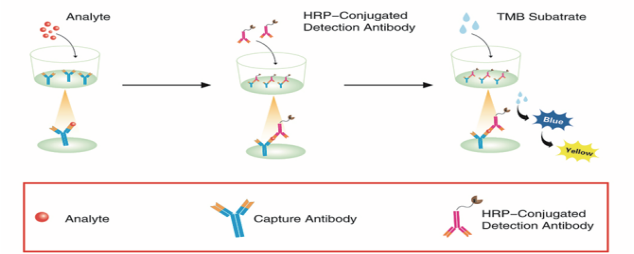
Sample Type	Average % of Expected Range(%)	Range(%)
Citrate plasma	98	85-101
Cell culture supernatants	100	87-106

BACKGROUND

CD86, also known as lymphocyte activation antigen B7-2(B70) , is a member of the cell surface Immunoglobulin superfamily. B7-2 exists primarily on the cell surface as a monomer and interacts with two co-stimulatory receptors, CD28 and cytotoxic T lymphocyte associated antigen 4(CTLA-4) , expressed on T cells, this induces signaling pathways that regulate T cell activation and tolerance, cytokine production, and CTL production. It is suggested that CD86-mediated contact between b-helper cells and t-helper cells promotes the proliferation and IgG secretion of normal b-cell and b-cell lymphomas. Recent studies have shown that CD86 also promotes the generation of APC maturation libraries and promotes APC function and survival. CD86 plays an important role in chronic hemodialysis, allergic lung inflammation, arthritis and antiviral response, so it is considered as a promising candidate for immunotherapy.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for CD86 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CD86 present is captured by the coated antibody after incubation. Following extensive washing, a HRP-conjugated antibody specific for CD86 is added to detect the captured CD86 protein in sample. The wells are then washed to remove unbound HRP-labled antibody and Tetramethyl-benzidine (TMB) reagent is added. Incubated at room temperature, only those wells that contain CD86, HRP-labled antibody will appear blue in color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450nm. The OD value is proportional to the concentration of Porcine CD86.



TECHNICAL HINTS AND LIMITATIONS

1.This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2.To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3.To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4.A thorough and consistent wash technique is essential for proper assay performance.
5.A standard curve should be generated for each set of samples assayed.
6.It is recommended that all standards and samples be assayed in duplicate.
7.Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8.In order to ensure the accuracy of the results, the standard curve should be made every time.

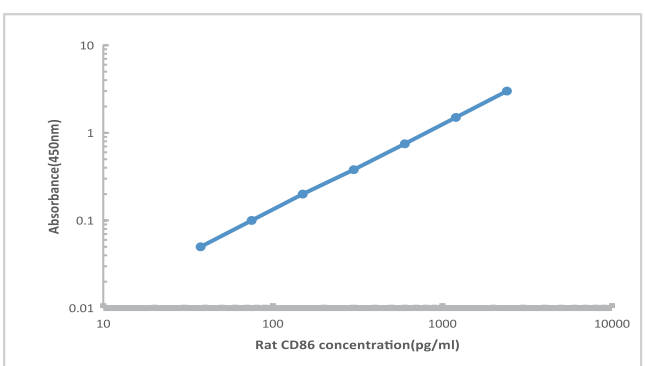
PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5.This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the CD86 ELISA

Standared(pg/ml)	OD.	OD.	Average	Corrected
0	0.048	0.056	0.052	-
37.50	0.172	0.153	0.162	0.110
75.0	0.250	0.222	0.236	0.184
150	0.372	0.330	0.351	0.299
300	0.591	0.525	0.558	0.506
600	0.960	0.852	0.906	0.854
1200	1.563	1.388	1.475	1.423
2400	2.546	2.261	2.404	2.352



Representative standard curve for CD86 ELISA.

ASSAY PROCEDURE

Prepare all reagents and standards as directed,wash the plate 3 times before the assay.

⇩

Add 100μl standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 120 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100μl working solution of HRP-Conjugate anti- Rat CD86 antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100μl Substrate solution to each well, incubate 10-30 minutes (depending on signal) at room temperature(25±2℃).Protect from light.

⇩

Add 50μl Stop solution to each well. Read at 450nm within 5 minutes.

Note: oscillatory reaction at room temperature 400r

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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 the graph.
4. The data may be linearized by plotting the log of the CD86 concentrations versus the log of the O.D. and the best fit line can be determined by

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate-antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	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**
Standard - lyophilized, 2400pg/ml upon reconstitution	2 vials	Store at -8°C**for six months
HRP -Congugated Antibody（100×）- 120 ul/vial	1 vial	Store at 2-8°C** for six months
Standard /sample Diluent - 16ml/vial	1 bottle	Store at 2-8°C** for six months
HRP Congugated Diluent - 16ml/vial	1 bottle	Store at 2-8°C** for six months
Wash Buffer Concentrate (20x) **-** 30 ml/vial	1 bottle	Store at 2-8°C** for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1.Microplate reader capable of measuring absorbance at 450 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.

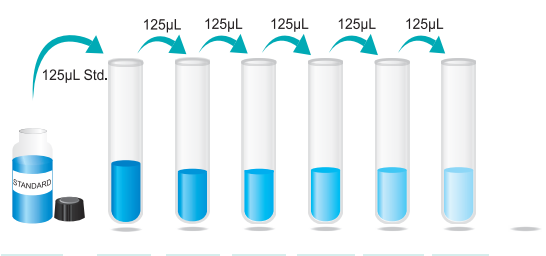
SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000g (or 3000rpm) to remove debris. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃.Centrifuge approximately for 15 minutes at 1000g(or 3000rpm). Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Plasma -Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000g(or 3000rpm) within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Note: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature(20-25℃) before use.
2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard**Sample - Reconstitute the Standard with 0.25mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 2400pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 125µL of Standard/Sample Diluent into 1200pg/ml tube and the remaining tubes. Use the stock solution of 2400pg/mL to produce a 2-folddilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 2400pg/mL standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard (0 pg/mL).
   ***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**
4. **Working solution of HRP-Congugated Antibody(100×):Make a 1:100 dilution in Reagent Diluent. If the entire 96-well plate is used, add 100uL of HRP Conjugate to 10mL of HRP-Congugated Diluent to make working dilution of HRP Conjugate and mix thoroughly prior to the assay. The rest of undiluted HRP Conjugate can be stored at 4°C for up to 6 months. DO NOT FREEZE**
   ***The working solution should be used within one day after dilution.**



Preparation of CD86 standard dilutions