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# **CUL9 ELISA Kit**





**Publications** 



## Overview

Quantity:	96 tests
Target:	CUL9
Reactivity:	Human
Method Type:	Sandwich ELISA
Application:	ELISA

#### Product Datails

Purpose:	Human PARC (CCL18) ELISA Kit for cell culture supernatants, plasma, and serum samples
Sample Type:	Plasma, Cell Culture Supernatant, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	The antibody pair provided in this kit recognizes human PARC / CCL18.
Sensitivity:	2 pg/mL
Characteristics:	<ul> <li>Strip plates and additional reagents allow for use in multiple experiments</li> <li>Quantitative protein detection</li> <li>Establishes normal range</li> <li>The best products for confirmation of antibody array data</li> </ul>
Components:	<ul> <li>Pre-Coated 96-well Strip Microplate</li> <li>Wash Buffer</li> <li>Stop Solution</li> <li>Assay Diluent(s)</li> </ul>

# **Product Details**

- · Lyophilized Standard
- Biotinylated Detection Antibody
- · Streptavidin-Conjugated HRP
- TMB One-Step Substrate

#### Material not included:

- · Distilled or deionized water
- Precision pipettes to deliver 2  $\mu$ L to 1  $\mu$ L volumes
- Adjustable 1-25 µL pipettes for reagent preparation
- 100 µL and 1 liter graduated cylinders
- Tubes to prepare standard and sample dilutions
- · Absorbent paper
- Microplate reader capable of measuring absorbance at 450nm
- · Log-log graph paper or computer and software for ELISA data analysis

# **Target Details**

Target:	CUL9
Alternative Name:	PARC (CUL9 Products)
Background:	PARC (Pulmonary and activation-regulated chemokine) belongs to the CC-Chemokines. PARC
	is referred to also as AMAC-1 (alternative activated macrophage associated CC-Chemokine),
	MIP-4 (macrophage inflammatory protein-4), or DC-CK1 (dendritic cell-derived chemokine-1).
	PARC is chemotactic for both activated CD3 (+) T-cells and non-activated CD14 (-)
	lymphocytes, but not for monocytes or granulocytes. The Human PARC ELISA (Enzyme-Linked
	Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the
	quantitative measurement of human PARC in serum, plasma, cell culture supernatants and
	urine. This assay employs an antibody specific for human PARC coated on a 96-well plate.
	Standards and samples are pipetted into the wells and PARC present in a sample is bound to
	the wells by the immobilized antibody. The wells are washed and biotinylated anti-human PARC
	antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated
	streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is
	added to the wells and color develops in proportion to the amount of PARC bound. The Stop
	Solution changes the color from blue to yellow, and the intensity of the color is measured at
	450 nm. Reproducibility: Intra-Assay: CV<10% Inter-Assay: CV<12%.
Gene ID:	6362
UniProt:	P55774

## **Application Details**

Application Notes:	Recommended Dilution for serum and plasma samples200 - 2,000 fold
Sample Volume:	100 μL
Plate:	Pre-coated
Protocol:	Prepare all reagents, samples and standards as instructed in the manual.
	2. Add 100 µL of standard or sample to each well.
	3. Incubate 2.5 h at RT or O/N at 4 °C.
	4. Add 100 μL of prepared biotin antibody to each well.
	5. Incubate 1 h at RT.
	6. Add 100 μL of prepared Streptavidin solution to each well.
	7. Incubate 45 min at RT.
	8. Add 100 µL of TMB One-Step Substrate Reagent to each well.
	9. Incubate 30 min at RT.
	10. Add 50 µL of Stop Solution to each well.
	11. Read at 450 nm immediately.

#### Reagent Preparation:

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. 2. Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) is used for dilution of serum/plasma samples, and Assay Diluent B (Item E) is used for dilution of culture supernatants and urine. 3. Assay Diluent B should be diluted 5-fold with deionized or distilled water. 4. Preparation of standard: Briefly spin the vial of Item C. Add 700 µl Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium/urine samples) into Item C vial to prepare a 50 ng/ml standard. Dissolve the powder thoroughly by a gentle mix. Add 40 µl PARC standard from the vial of Item C, into a tube with 960 µl Assay Diluent A or 1x Assay Diluent B to prepare a 2000 pg/ml stock standard solution. Pipette 400 µl Assay Diluent A or 1x Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/ml). 5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer. 6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 100-fold with 1x Assay Diluent B and used in step 4 of Part VI Assay Procedure. 7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 30,000-fold with 1x Assay Diluent B. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 2 µl of HRP-Streptavidin concentrate into a tube with 198.0 µl 1x Assay Diluent B to

Assay Procedure:

prepare a 100-fold diluted HRP-Streptavidin solution (do not store the diluted solution for next day use). Mix through and then pipette 50 µl of prepared 100-fold diluted solution into a tube with 15 ml 1x Assay Diluent B to prepare a final 30,000 fold diluted HRP-Streptavidin solution. 1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate. 2. Add 100 µl of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4°C with gentle shaking. 3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette 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. 4. Add 100 µl of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking. 5. Discard the solution. Repeat the wash as in step 3. 6. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking. 7. Discard the solution. Repeat the wash as in step 3. 8. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to

Calculation of Results:

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking. 9. Add

50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

Restrictions:

For Research Use only

6 months

## Handling

Storage: -20 °C

Storage Comment: The entire kit may be stored at -20 °C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4 °C for up to 6 months. For extended storage, it is recommended to store at -80 °C.

#### **Publications**

**Expiry Date:** 

Product cited in:

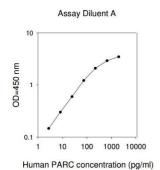
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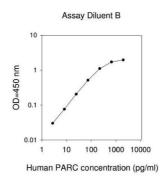
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# **Images**





#### **ELISA**

Image 1.