Human uPAR ELISA Assay Kit

Catalog Number:
PAR31-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0 (20 AUG 24)

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INTENDED USE

The Eagle Biosciences Human uPAR ELISA Assay Kit is intended for the quantitative measurement of uPAR in human serum/plasma, urine, and more. The UPAR ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

REAGENTS PROVIDED

Content	Volume
CP (Coated Plate)	96 well
S (Standard)	9 vial
DA (Detect Antibody)	6 mL/bottle
SH (Streptavidin-HRP)	12 mL/bottle
AB (Assay Buffer 1x)	12 mL/bottle
TS (TMB Substrate)	12 mL/bottle
SS (Stop Solution)	12 mL/bottle
WB (Wash Buffer 10x)	50 mL/bottle
SF (Sealer Film)	6 pieces

Note: After the kit is opened, the stabilization period of each content is 30 days, so please use the kit within 30 days after opening.

REAGENT PREPARATION

Washing Buffer (1x) Preparation:

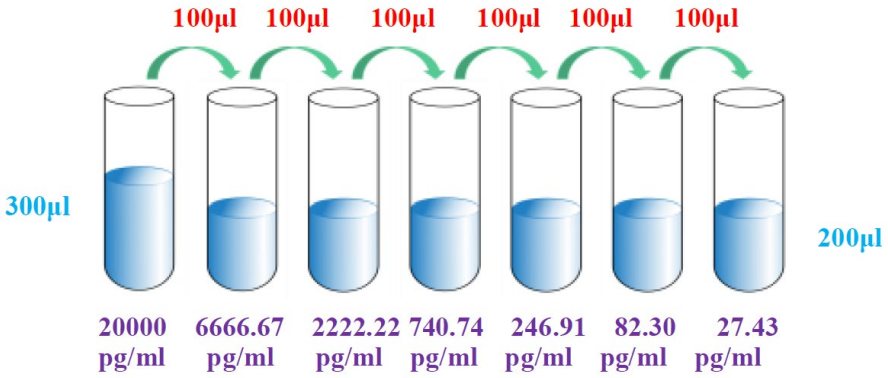
Pour entire contents (50 mL) of the **Washing Buffer Concentrate** (10x) into a clean 500 mL graduated cylinder. Bring to final volume of 500 mL with glass-distilled or deionized water. Transfer to a clean wash bottle and store at 2 to 25°C.

Standard Curve Preparation:

S1 to S7 and S0 are ready to use for serum and plasma.

For other sample types, prepare the standard curve with whatever buffer (SPB, Sample Prepared Buffer) is used to prepare the sample, such as cell culture supernatant, tissue grinding liquid, cell lysate, etc. For urine samples use AB (Assay Buffer) to prepare the standard curve.

The Human uPAR Standard 200,000 pg/mL 30 µL + 270 µL SPB serves as the high standard (20,000 pg/mL). Pipette 200 µL of SPB into each tube. Use the high standard to produce a 1:2 dilution series. Mix each tube thoroughly before the next transfer. SPB serves as the zero standard (0 pg/mL).

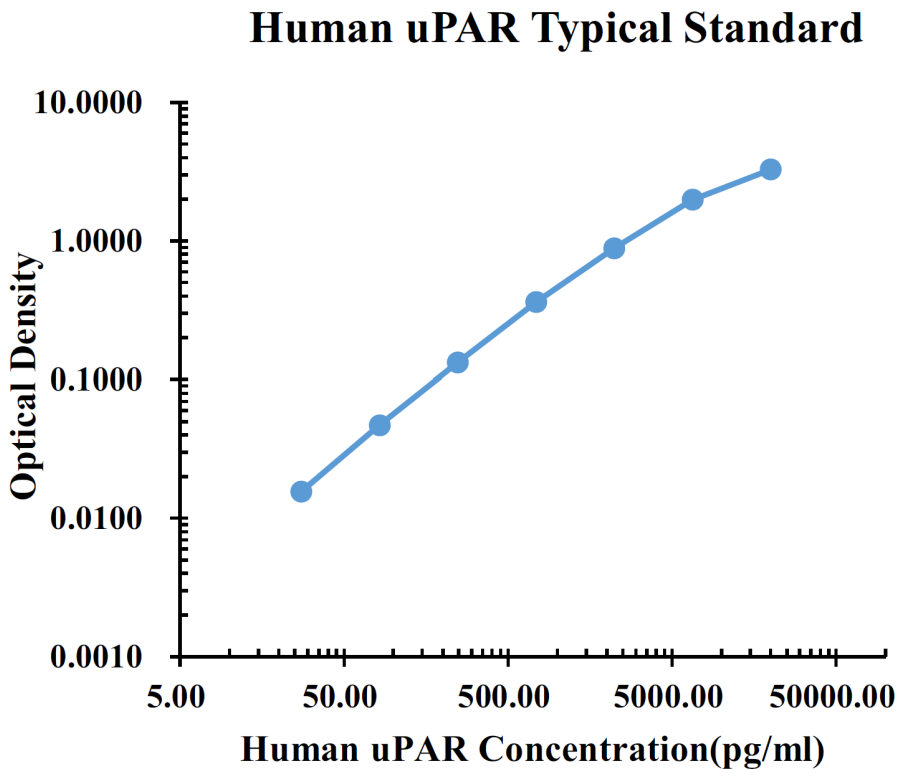


ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess **CP (Coated Plate)** strips from the plate frame, return them to the foil pouch and reseal.
3. Add 50 µL of **AB (Assay Buffer)** to each well.
4. Add 50 µL of **Standard or Sample** per well. Ensure reagent addition is uninterrupted and completed withing 15 minutes.
5. Add 50 µL of **DA (Detect Antibody)** to each well.
6. Cover with an **SF (Sealer Film).** Incubate at room temperature (18 to 25ºC) for 1 hour on a microplate **shaker** set to 500 rpm.
7. Aspirate each well and **wash**, repeating the process four times. Wash by filling each well with WB (Washing Buffer 300 µL). Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining WB (Washing Buffer) by aspirating or decanting. Invert the plate and **blot** it against clean paper towels.
8. Add 100 µL of **SH (Streptavidin-HRP)** to each well.
9. Cover with a new **SF (Sealer Film).** Incubate at room temperature (18 to 25ºC) for 15 minutes on a microplate **shaker** set at 500 rpm.
10. Repeat aspiration/**wash** as in step 7.
11. Add 100 µL of **TS (TMB Substrate)** to each well. Incubate for 5-30 minutes at room temperature.
12. Add 100 µL of **SS (Stop Solution)** to each well.
13. Determine the optical density within 30 minutes, using a microplate **reader** set to 450 nm corrected with 570 nm or 630 nm.

TYPICAL DATA



pg/mL	O.D.		Average	Corrected
0.00	0.0148	0.0166	0.0157	
27.43	0.0262	0.0238	0.0250	0.0093
82.30	0.0453	0.0491	0.0472	0.0315
246.91	0.1061	0.1035	0.1048	0.0891
740.46	0.2701	0.2723	0.2712	0.2555
2222.22	0.7935	0.7777	0.7856	0.7699
6666.67	2.0410	2.0280	2.0345	2.0188
20000.00	3.9180	3.9010	3.9095	3.8938

SENSITIVITY

The minimum detectable dose (MDD) of human uPAR is typically less than 8.79 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of ten zero standard replicates and calculating the corresponding concentration.

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)

	Intra-assay Precision			Inter-assay Precision		
Sample Number	S1	S2	S3	S1	S2	S3
	22	22	22	6	6	6
Average (pg/mL)	370.1	1850.1	6148.8	356.8	1784.4	6135.0
Standard Deviation	10.2	87.9	204.1	18.4	88.2	389.3
Coefficient of variation (%)	2.8	4.8	3.3	5.2	4.9	6.3

RECOVERY

The spike recovery was evaluated by spiking 3 levels of human uPAR into healthy human serum samples. The un-spiked serum was used as a blank in this experiment. The recovery ranged from 80% to 103% with an overall mean recovery of 96%.

LINEARITY

To assess the linearity of the assay, five samples were spiked with high concentration of uPAR in human serum and diluted with Sample Diluent to produce samples with values within the dynamic range of the assay. The linearity ranged from 81% to 91% with an overall mean recovery of 86%.

SAMPLE VALUES

Serum/Plasma - Thirty samples from apparently healthy volunteers were evaluated for the presence of UPARin this assay. No medical histories were available for the donors.

Sample Matrix	Sample Evaluated	Range (pg/mL)	Detectable %	Mean of Detectable (pg/mL)
Serum	30	1694.27 - 8807.19	100%	3895.95

n.d. = non-detectable. Samples measured below the sensitivity are considered to be non-detectable.

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