Rat ICAM-1 ELISA Instructions

Cat:ERY0011

Content

	CAT	Volume
❶ CP (Coated Plate)	ERY0011CP	96 well
❷ S (Standard)	ERY0011S1	2 vial
❸ SD (Sample Diluent)	ESD02	15 ml/bottle
❹ DD (Detect Antibody Diluent)	EDD02	6 ml/bottle
❺ 100×DA-H (Detect Antibody)	ERY0011DA-H	50μl/vial
❻ AB (Assay Buffer 1×)	EAB01	12 ml/bottle
❼ TS (TMB Substrate)	ETS01	12 ml/bottle
❽ SS (Stop Solution)	ESS01	12 ml/bottle
❾ WB (Wash Buffer 10×)	EWB01	50 ml/bottle
❿ SF (Sealer Film)	ESF01	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 (1×) Preparation

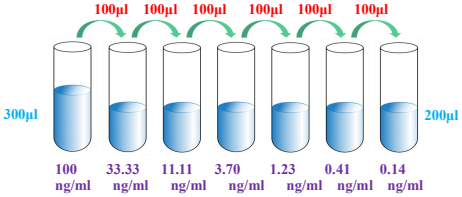
Pour entire contents (50 ml) of the **Washing Buffer Concentrate** (10×) into a clean 500 ml graduated cylinder. Bring to final volume of 500 ml with glass-distilled or deionized water. Transfer to a clean wash bottle and store at 2 to 25℃.

Standard Curve Preparation:

Reconstitute rat ICAM-1 Standard by addition of distilled water as S1. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 100ng/ml).

Allow the standard to reconstitute for 10-30 minutes. Mix well prior to making dilutions.

The rat ICAM-1 Standard ERY0011S1 as the high standard (100ng/ml). Pipette 200 μl of **SD** (Sample Diluent) into each tube. Use the high standard to produce a 1:2 dilution series. Mix each tube thoroughly before the next transfer. **SD** (Sample Diluent) serves as the zero standard (0 ng/ml).



1×DA-H Preparation:

Mix well prior to making dilutions.

Make a 1:100 dilution of the concentrated Detect Antibody solution with **DD** (Detect Antibody Diluent) in a clean plastic tube as needed according to the Standards and Samples.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

❶ Prepare all reagents and working standards as directed in the previous sections.

❷ Remove excess **CP** (Coated Plate) strips from the plate frame, return them to the foil pouch and reseal.

❸ Add 50 μl of **AB** (Assay Buffer) to each well.

❹ Add 50 μl or10 μl of **Standard or sample** per well. Ensure reagent addition is uninterrupted and completed within 15 minutes.

❺ Add 50 μl of **DA-H** (Detect Antibody) to each well.

❻ Cover with an SF (Sealer Film). Incubate at room temperature (18 to 25℃) for 30 min on a microplate shaker set at 500 rpm.

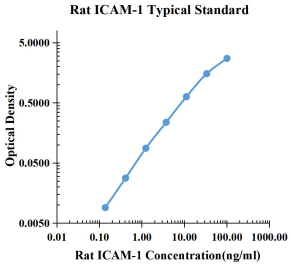
❼ Aspirate each well and wash, repeating the process four times. Wash by filling each well with WB (Washing Buffer 300 μl). Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining WB (Washing Buffer) by aspirating or decanting. Invert the plate and blot it against clean paper towels.

❽ Add 100 μl of TS(TMB Substrate) to each well. Incubate for 5-30 minutes at room temperature.

❾ Add 100 μlofSS(Stop Solution) to each well.

❿ Determine the optical density within 30 minutes, using microplate reader set to 450 nm corrected with 570 nm or 630 nm

TYPICAL DATA



ng/ml	O.D.		Average	Corrected
0.00	0.0060	0.0064	0.0062	
0.14	0.0153	0.0153	0.0153	0.0091
0.41	0.0352	0.0335	0.0344	0.0282
1.23	0.0946	0.0942	0.0944	0.0882
3.70	0.2485	0.2398	0.2442	0.2380
11.11	0.6446	0.6379	0.6413	0.6351
33.33	1.5470	1.5320	1.5395	1.5333
100.00	2.7720	2.7450	2.7585	2.7523

SENSITIVITY

The minimum detectable dose (MDD) of rat ICAM-1 is typically less than 0.04 ng/ml (50 µl of sample volume) or 0.07 ng/ml (10 µl of sample volume).

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-two 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（ng/ml）	4.3	17.2	45.8	4.5	17.8	48.5
Standard Deviation	0.1	0.3	1.7	0.1	0.4	0.9
Coefficient of Variation（%）	2.7	1.9	3.7	3.1	2.5	1.9

RECOVERY

The spike recovery was evaluated by spiking 3 levels of rat ICAM-1 into health rat serum sample. The un-spiked serum was used as blank in this experiment.

The recovery ranged from 80% to 118% with an overall mean recovery of 105%.

LINEARITY

To assess the linearity of the assay, five samples were spiked with high concentration of ICAM-1 in rat serum and diluted with Sample Diluent to produce samples with values within the dynamic range of the assay.

The linearity ranged from 83% to 116% with an overall mean recovery of 99%.

SAMPLE VALUES

Serum/Plasma – Thirty samples from apparently healthy rats were evaluated for the presence of rat ICAM-1 in this assay. No medical histories were available for the donors.

Sample Matrix	Sample Evaluated	Range (ng/ml)	Detectable %	Mean of Detectable (ng/ml)
Serum	30	13.35-34.28	100	22.48

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