

Human ICAM-1 / CD54 ELISA Set

Catalog No. CDK097A, B, C, D

Quantity: 5, 10, 15, 20 x 96 tests

Specificity:	Native and recombinant human soluble ICAM-1
Sensitivity:	< 0.1 ng/ml
Range:	0.25 – 8.0 ng/ml
Sample Type:	Cell supernatants, buffered solutions, serum, plasma samples and other bodily fluids.
Cross-Reactivity:	No cross reactivity with other adhesion molecules.

1. INTENDED USE

The Cell Sciences® Human ICAM-1/CD54 ELISA Set is intended for use in a 'do it yourself' solid phase sandwich ELISA for the *in-vitro* qualitative and quantitative determination of IFN- γ in supernatants, buffered solutions, serum, plasma samples and other body fluids. This assay will recognize both native and recombinant human ICAM-1.

This kit has been configured for research use only.

2. PRINCIPLE OF THE METHOD

A capture antibody highly specific for ICAM-1 is coated to the wells a microtiter strip plate. Binding of ICAM-1 samples and known standards to the capture antibodies and subsequent binding of the biotinylated anti-ICAM-1 secondary antibody to the analyte is completed during the same incubation period. Any excess unbound analyte and secondary antibody is removed.

Next HRP conjugate solution is added to every well including the zero wells. Following incubation, excess conjugate is removed by careful washing. A chromogen substrate is added to the wells resulting in the progressive development of a blue colored complex with the conjugate. The color development is then stopped by the addition of acid turning the resultant final product yellow. The intensity of the produced colored complex is directly proportional to the concentration of CD54 present in the samples and standards.

The absorbance of the color complex is then measured and the generated OD values for each standard are plotted against expected concentration forming a standard curve. This standard curve can then be used to accurately determine the concentration of CD54 in any sample tested.



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3. REAGENTS PROVIDED AND RECONSTITUTION

REAGENTS (Store at 2-8°C)	5 x 96 wells	10 x 96 wells	15 x 96 wells	20 x 96 wells	RECONSTITUTION * STORAGE (see Section 9 – Assay Preparation)
A. ICAM-1 Standard 8 ng/ml	5 vials	10 vials	15 vials	20 vials	Reconstitute as directed on the vial. <i>Discard reconstituted standard after use.</i>
B. Anti-ICAM-1 Capture Antibody (0.5 ml/ vial)	1 vial	2 vials	3 vials	4 vials	Sterile. Dilute prior to use. <i>Discard diluted antibody after use.</i>
C. Biotinylated anti-ICAM-1 Detection Antibody (lyophilized)	1 vial	2 vials	3 vials	4 vials	Reconstitute with 0.55 ml Reconstitution Buffer prior to use. <i>Store reconstituted detection antibody at 2-8°C for 1 year.</i>
D. Streptavidin-HRP (25 µl/ vial)	1 vial	2 vials	3 vials	4 vials	Dilute prior to use. <i>Discard diluted Streptavidin-HRP conjugate after use.</i>
E. TMB Substrate (25 ml/ vial)	2 vials	4 vials	6 vials	8 vials	Ready-to-use.

4. MATERIAL REQUIRED BUT NOT PROVIDED

- 96-well Microtiter plates (e.g. Nunc Maxisorp Cat # 468667)
- Reconstitution Buffer (1x PBS + 0.09% Azide). *Store at 2-8°C for up to 1 week.*
- Coating Buffer (1x PBS, pH 7.2-7.4). *Store at 2-8°C for up to 1 week.*
- Wash Buffer (1x PBS + 0.05% Tween20). *Use immediately.*
- Blocking Buffer (1x PBS + 5% BSA). *Store at 2-8°C for up to 1 week.*
- Standard and Detection Antibody Dilution Buffer (1x PBS, 1% BSA). *Store 2-8°C for up to 1 week.*
- HRP Diluent Buffer (1x PBS + 1% BSA + 0.1% Tween20). *Store at 2-8°C for up to 1 week.*
- Stop Reagent (1M Sulfuric Acid)
- Microtiter plate reader with appropriate filters (450nm required + optional 620nm reference filter)
- Microplate washer or wash bottle
- 10, 50, 100, 200 and 1,000 µl adjustable single channel micropipettes with disposable tips
- 50-300 ml multi-channel micropipette with disposable tips
- Multichannel micropipette reagent reservoirs
- Distilled water
- Vortex mixer
- Miscellaneous laboratory plastic and/or glass, if possible sterile.



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5. STORAGE INSTRUCTIONS

Store the kit reagents between 2 and 8°C. Immediately after use, remaining reagents should be returned to cold storage (2-8°C). The expiration date of the kit and reagents is stated on box front labels. The expiry date of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

Reconstituted Biotinylated anti- ICAM-1 Detection Antibody: Store at 2-8°C for up to 1 year.

Reconstituted ICAM-1 Standard: Discard after use.

6. SAFETY AND PRECAUTIONS FOR USE

- Handling of reagents, serum, or plasma specimens should be in accordance with local safety procedures, e.g.CDC/NIH Health manual: "Biosafety in Microbiological and Biomedical Laboratories" 1984.
- Laboratory gloves should be worn at all times.
- Avoid any skin contact with H₂SO₄ and TMB. In case of contact, wash thoroughly with water.
- Do not eat, drink, smoke or apply cosmetics where kit reagents are used.
- Do not pipette by mouth.
- When not in use, kit components should be stored refrigerated or frozen, as indicated on vial or bottle labels.
- All reagents should be warmed to room temperature before use. Lyophilized standards should be discarded after use.
- Cover or cap all reagents when not in use.
- Do not mix or interchange reagents between different lots.
- Do not use reagents beyond the expiration date of the kit.
- Use a clean, disposable, plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross contamination. For the dispensing of H₂SO₄ and substrate solution. Avoid pipettes with metal parts.
- Use a clean plastic container to prepare the washing solution.
- Thoroughly mix the reagents and samples before use by agitation or swirling.
- All residual washing liquid must be drained from the wells by efficient aspiration, or by decantation, followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
- The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent color development. **Warning: TMB is toxic. Avoid direct contact with hands. Dispose of properly.**
- If a dark blue color develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbances within 1 hour after completion of the assay.
- When pipetting reagents, maintain a consistent order of addition from well to well. This will ensure equal incubation times for all wells.
- Follow incubation times described in the assay procedure.
- Dispense the TMB solution within 15 min of the washing of the microtiter plate.



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7. SPECIMEN COLLECTION, PROCESSING & STORAGE

Cell culture supernatants, human serum, plasma or other biological samples are suitable for use in the assay. Remove serum from the clot or red cells respectively, as soon as possible after clotting and separation.

Cell culture supernatants: Remove particulates and aggregates by centrifugation at 1000 x g for 10 min.

Serum: Use pyrogen/endotoxin free collecting tubes. Serum should be removed rapidly and carefully from the red cells after clotting. After clotting, centrifuge at approximately 1000 x g for 10 min and remove serum.

Plasma: EDTA, citrate and heparin plasma can be assayed. Spin samples at 1000 x g for 30 min. to remove particulates. Harvest plasma.

Storage: If not analyzed shortly after collection, samples should be aliquoted (250-500 µl) to avoid repeated freeze-thaw cycles and stored frozen at -80°C. Avoid multiple freeze-thaw cycles of frozen specimens.

Recommendation: Do not thaw by heating at 37°C or 56°C. Thaw at room temperature, and make sure that the sample is completely thawed and homogeneous before use. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particles are present, these should be removed prior to use by centrifugation or filtration.

8. PLATE PREPARATION:

For 1 96-well plate, add 100 µl of Capture Antibody to 10 ml of Coating Buffer. *Discard diluted antibody after use.*

1.	Addition	Add 100 µl of diluted Capture Antibody to each well. <i>Discard diluted antibody.</i>
2.	Incubation	Cover with a plastic plate cover and incubate at 4°C overnight.
3.	Wash	Remove the cover and wash the plate as follows: a) Aspirate the liquid from each well. b) Dispense 0.4 ml of Wash Solution into each well. c) Aspirate the contents of each well. d) Repeat steps b and c.
4.	Addition	Add 250 µl of Blocking Buffer to each well.
5.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for 2 hours.
6.	Wash	Remove the cover and wash the plate as follows: a) Aspirate the liquid from each well. b) Dispense 0.4 ml of Wash Solution into each well. c) Aspirate the contents of each well. d) Repeat steps b and c twice more.

For immediate use of the plate, continue to Section 9.

To store the coated and blocked plates for future use, dry the plate on the bench at room temperature for 24 hours. Cover the plates and store at 2-8°C in a sealed plastic bag with desiccant for up to 12 months



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9. ASSAY PREPARATION

Bring all reagents to room temperature before use.

Determine the number of microwell strips required to test the desired number of samples, plus appropriate number of wells needed for running zeros and standards. Each sample, standard and zero should be tested **in duplicate**.

Example plate layout (example shown for a 6-point standard curve)

	Standards (ng/ml)		Sample Wells									
	1	2	3	4	5	6	7	8	9	10	11	12
A	8	8										
B	4	4										
C	2	2										
D	1	1										
E	0.5	0.5										
F	0.25	0.25										
G	zero	zero										
H												

All remaining empty wells can be used to test samples in duplicate.

Preparation of Standard

Standard vials must be reconstituted with the volume of standard diluent shown on the vial immediately prior to use. This reconstitution gives a stock solution of 8 ng/ml of ICAM-1. **Mix the reconstituted standard gently by inversion only**. Serial dilutions of the standard are made directly in the assay plate to provide the concentration range from 8 to 0.25 ng/ μ l. A fresh standard curve should be produced for each new assay.

- Immediately after reconstitution add 200 μ l of the reconstituted standard to wells A1 and A2, which provides the highest concentration standard at 8 ng/ml.
- Add 100 μ l of Standard Diluent to the remaining standard wells B1 and B2 to F1 and F2.
- Transfer 100 μ l from wells A1 and A2 to B1 and B2. Mix the well contents by repeated aspirations and ejections taking care not to scratch the inner surface of the wells.
- Continue this 1:1 dilution using 100 μ l from wells B1 and B2 through to wells F1 and F2, providing a serial diluted standard curve ranging from 8 ng/ml to 0.25 pg/ml.
- Discard 100 μ l from the final wells of the standard curve (F1 and F2).

Alternatively, these dilutions can be performed in separate clean tubes and immediately transferred directly into the relevant wells.



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Preparation Biotinylated anti-ICAM-1 Detection Antibody

It is recommended this reagent is prepared immediately before use. Dilute the reconstituted biotinylated anti-ICAM-1 with the Biotinylated Antibody Diluent in an appropriate clean glass vial.

For one plate, add 100 μ l of the reconstituted Detection Antibody into 5 mL of Biotinylated Antibody dilution buffer.

Preparation of Streptavidin-HRP

It is recommended to centrifuge vial for a few seconds in a microcentrifuge to collect all the volume at the bottom.

Dilute 5 μ l of Streptavidin-HRP into 0.5 ml of HRP diluent buffer immediately before use.

Add 150 μ l of the diluted HRP solution into 10 mL of HRP diluent buffer.

Do not keep these solutions for future experiments.

10. ASSAY METHOD

We strongly recommend that every vial is mixed thoroughly without foaming prior to use, except the standard vial, which must be mixed gently by inversion only.

Note: Final preparation of Biotinylated Anti-ICAM-1 (Section 9.3) and Streptavidin-HRP (Section 9.4) should occur immediately before use.

**Incubation time of the substrate solution is usually determined by the ELISA reader performance.*

Assay Step		Details
1.	Preparation	Prepare Standard curve as shown in section 9.2 above.
2.	Addition	Add 100 μ l of each standard, sample and zero (Standard Dilution Buffer) in duplicate to appropriate number of wells.
3.	Addition	Add 50 μ l of diluted Detection Antibody to all wells.
4.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for 1 hour .
5.	Wash	Remove the cover and wash the plate as follows: a) Aspirate the liquid from each well b) Dispense 0.4 ml of washing solution into each well c) Aspirate the contents of each well d) Repeat step b and c
6.	Addition	Add 100 μ l of Streptavidin-HRP solution into all wells.
7.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for 30 min .
8.	Wash	Repeat wash step 5.
9.	Addition	Add 100 μ l of ready-to-use TMB Substrate Solution into all wells.
10.	Incubation	Incubate in the dark for 5-15 minutes* at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.
11.	Addition	Add 100 μ l of H₂SO₄: Stop Reagent into all wells.



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Read the absorbance value of each well (immediately after step 11.) on a spectrophotometer using 450 nm as the primary wavelength and optimally 620 nm as the reference wave length (610 nm to 650 nm is acceptable).

Many ELISA readers only record absorbance up to 2.0 O.D. Therefore, the color development within individual microwells must be observed by the analyst, and the substrate reaction stopped before positive wells are no longer within recordable range.

11. DATA ANALYSIS

Calculate the average absorbance values for each set of duplicate standards, controls and samples. Ideally duplicates should be within 20% of the mean.

Generate a linear standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding ICAM-1 standard concentration on the horizontal axis.

The amount of ICAM-1 in each sample is determined by extrapolating OD values against ICAM-1 standard concentrations using the standard curve.

12. ASSAY LIMITATIONS

Do not extrapolate the standard curve beyond the maximum standard curve point. The dose-response is non-linear in this region, and good accuracy is difficult to obtain. Concentrated samples above the maximum standard concentration must be diluted with Standard diluent or with your own sample buffer to produce an OD value within the range of the standard curve. Following analysis of such samples, always multiply results by the appropriate dilution factor to produce actual final concentration.

The influence of various drugs on end results has not been investigated. Bacterial or fungal contamination and laboratory cross-contamination may also cause irregular results.

Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Washing Buffer, fill with Washing Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.

As with most biological assays conditions may vary from assay to assay therefore **a fresh standard curve must be prepared and run for every assay.**

13. PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity or minimum detectable dose of this ICAM-1 ELISA Set was determined using the Cell Sciences ICAM-1 ELISA kit (which contains the same antibodies) and was found to be <0.1 ng/ml. This was determined by adding 3 standard deviations to the mean OD obtained when the zero standard was assayed 32 times.

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