

PRODUCT INFORMATION SUMMARY**Human ICAM-1
"SUPER X" ELISA Kit**

Product Number **RHF717CKX**
 5 Plate Kit (5 x 96 tests)
RHF717CKX2 (2 x 96 tests)
RHF717CKX1 (96 tests)

Items Provided:

PRE-COATED ELISA Plates (Single, 2 or 5 plates)
 Biotin-Labeled tracer 25.0 ug or 0.5 mL (see vial)
 Antigen Standard 10.0 ug or 1.0 ug (see vial)
 Streptavidin-HRP 0.5 mL
 TMB Substrate 25 mL x 2 (15 mL - 2 plate)
 Wash Buffer Concentrate 125 mL (20X) (50 mL - 2 plate)

DESCRIPTION:

Human ICAM-1 **SUPER X ELISA Kit** provides single, two or five **PRE-COATED** microplates (coated with antigen affinity purified capture antibody stabilized with our proprietary ELISA coating/blocking reagent. A biotin labeled tracer antibody, antigen standard, HRP developing reagents and wash buffer are included.

Note: Reconstitute components only when ready to run assay.

TRACER ANTIBODY:

Provided as 25 ug (lyophilized) or as 0.5 mL liquid @ 50.0 ug/mL - **see vial-** of Biotin labeled, antigen-affinity purified antibody, additive-free. For lyophilized : Reconstitute in 500 uL sterile water **containing 0.1% BSA.** (FREEZE aliquots for long-term storage).

For vial provided as **Liquid - Store refrigerated only - contains preservative**

STANDARD: Provided as 10.0 ug or 1.0 ug (see vial) of recombinant Human ICAM-1. Quick-spin and **reconstitute in 100 uL sterile or distilled water** (pH 8.0) - Further dilutions can be made in 0.05% Tween-20, 0.1% BSA in PBS.

DEVELOPING REAGENTS:

- Streptavidin-HRP (S100180CX) 0.5 mL - **store @ -20 Deg. C.**
- TMB Substrate Solutions - Part A and Part B (25.0 mL each) cat # ES200CX
- Wash Buffer Concentrate (20X concentrate) Mix 1 volume of wash buffer with 19 volumes of distilled water. Stable for one month @ 4 Deg. C. once mixed to working volume.
- Diluent (10X) -Dilute 1 part with 9 parts distilled water
- **STOP Solution (1X)** 2N sulfuric acid - **CAUTION: CAUSTIC**

HANDLING/ STORAGE: Reconstitute reagents when ready to build ELISA assay. Tracer Antibody provided as lyophilized - after reconstitution be stored for approximately one month at 4 Degrees C. Or store **frozen** at -20 Degrees C. for up to 6 months.

Biotin tracer provided as 0.5 mL liquid- STORE refrigerated only.

Standard (rec. ICAM-1) can be stored in liquid state (@ 4 Deg. C.) For up to one week, or store **frozen, with addition of 0.1% BSA,** at -20 Deg. C. for up to 2 months. AVOID repeat freeze-thaw. Pre-coated ELISA plates should be stored in sealed plastic bag with desiccant pack, and are stable until expiration date on kit box.

Store Streptavidin-HRP (S100180X) **frozen** @ -20 Deg. C.

Store TMB solutions and wash buffer concentrate at 4 Deg. C.
CAUTION: Substrate Solution B contains 20% acetone. FLAMMABLE.
Keep away from sources of heat or flame.

MATERIALS RECOMMENDED:

Tween -20.

BSA (ELISA grade only, protease-free)

Dubelco's PBS (10X)

PBS: Dilute to 1XPBS in sterile water

Diluent: use ANTIGENIX ED100 (10X) (if supplied) :
Or 0.05% Tween-20, 0.1% BSA in PBS

PLATE PREPARATION:

Plates are **pre-coated** with capture antibody and blocked/stabilized with ANTIGENIX proprietary ELISA coating stabilizer and are **ready to use!**

Store plates refrigerated (@ 4 Deg. C.) in sealed plastic bags with desiccant pack.

PROTOCOL:

STANDARD/SAMPLE: Dilute a portion of the standard (store unused standard in aliquots, high concentration, frozen -20 Deg. C. with addition of 0.1% BSA) from **2.0 ng/mL** to zero in diluent (8 point , 1:2 serial dilution). Immediately add 100 uL of standard or sample to each well in duplicate. Incubate at room temp. for **90 minutes.**

DETECTION: Aspirate and wash plate 4 times. **Dilute** detection (Tracer) antibody in diluent to concentration of **0.10 ug/mL.** (Approx. 1:500 from 50.0 ug/mL) **Add** 100 uL per well.
Incubate at room temperature for **30 minutes.**

STREPTAVIDIN-HRP: Aspirate and wash plate 4 times. **Dilute** Streptavidin-HRP conjugate approx. **1:2,500** in diluent. = 2.0 uL HRP to 5.0 mL Diluent. (May need to optimize) **Add** 100 uL per well, **incubate 30 minutes** at room temperature.

SUBSTRATE: Prepare substrate solution no more than 15 minutes before last incubation of assay: **Mix** one part TMB Solution A with one part TMB Solution B in a clean container. If, upon mixing, TMB solution turns blue - TMB solution is **contaminated- DO NOT USE**. Use mixed substrate solution **WITHIN 2 Hours, and AVOID DIRECT LIGHT**.

Aspirate and wash plate 4 times. **Note:** Wash steps are critical! **Add** 100 uL **substrate solution** to each well. **Incubate** at room temp. for color development. **Add** 100 uL of **Stop solution** (2N Sulfuric Acid) within **10 minutes** to stop color development - gently tap plate to mix. Read plate at 450 nm within 30 minutes of addition of stop solution.

NOTE: reliable standard curves are obtained when O.D. readings do not exceed 0.30 units for the zero standard concentration, or 2.0 units for the highest standard concentration.

X-Reactivity data:

No measurable X-reactivity was observed @ 40-50 ng/mL with following factors:

Human: IFN Gamma, PE-CAM, VCAM-1, TNF alpha, IL-1 beta

RESEARCH USE ONLY -NOT For DIAGNOSTIC USE

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