HUMAN CALRETICULIN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN CALRETICULIN CONCENTRATIONS IN SERUM AND EDTA PLASMA

PURCHASE INFORMATION:

ELISA NAME	HUMAN CALRETICULIN ELISA
Catalog No.	SK00016-01
Lot No.	
Formulation	96 T
Standard range	3.9 - 500 ng/mL
Sensitivity	1 ng/mL
Sample Volume	100 µl
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Sample Type	Serum, EDTA Plasma
Specificity	Human CALRETICULIN only
Intra-assay Precision	4 - 8%
Inter-assay Precision	8 - 12%
Storage	2 - 8°C

FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTRODUCTION

Human CALRETICULIN immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human CALRETICULIN in serum and EDTA plasma. It contains recombinant human CALRETICULIN and antibodies raised against this protein. It has been shown to accurately quantify recombinant human CALRETICULIN. Results obtained with naturally occurring CALRETICULIN samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human CALRETICULIN.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for CALRETICULIN has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CALRETICULIN present is bound by the immobilized antibody. After washing away any unbound substances, an antibody specific for CALRETICULIN is added to the wells. Following a wash to remove any unbound antibody reagent, Anti Rabbit IgG HRP conjugate is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of CALRETICULIN bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute reagents with those from other lots or sources.

_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_ If samples generate values higher than the highest standard, dilute the samples with dilution buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
CALRETICULIN Microplate – 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against CALRETICULIN.	**016-01-01**	**1 plate**
CALRETICULIN Standard – 500 ng/vial of recombinant human CALRETICULIN in a buffered protein base with preservatives; lyophilized.	**016-01-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrated of an antibody against CALRETICULIN with preservatives; lyophilized.	**016-01-03**	**1 vial**
Positive Control – one vial of recombinant CALRETICULIN, lyophilized	**016-01-04**	**1 vial**
Anti Rabbit IgG-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Goat anti Rabbit IgG conjugate to HRP	**ARIGHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservatives	**DB06**	**1 bottle**
ARIGHRP Diluent Solution - 12mL of buffered protein based solution with preservatives.	**DB08**	**1 bottle**
Wash Buffer – 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution – 11 mL of TMB substrate solution	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.5M HCl	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 6 months. For longer storage, unopened Standard, Positive

Control, Detection Antibody Concentrate should be stored at -20°C or -70°C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard and Detection Antibody Concentrated Solution SHOULD BE STORED at -20 °C or -70°C for up to one month. Anti Rabbit IgG-HRP Conjugate 100-fold concentrated solution **(protect from light)** and other components may be stored at 2 - 8°C for up to 6 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack and seal along the entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C after opening.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

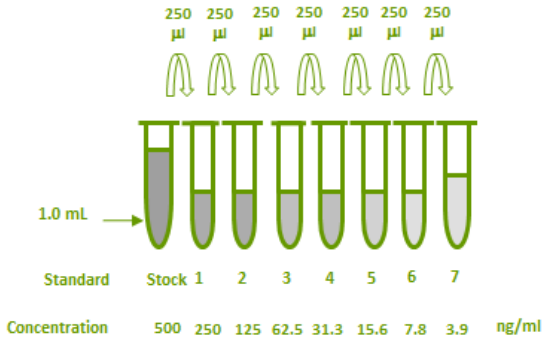
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.

CALRETICULIN Standard - Refer to vial label for reconstitution volume. Reconstitute the **CALRETICULIN** standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 500 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **500 ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1.0 ml	500 ng/ml
# 1	250µl of stock	250µl	250 ng/ml
# 2	250µl of 1	250µl	125 ng/ml
# 3	250µl of 2	250µl	62.5 ng/ml
# 4	250µl of 3	250µl	31.3 ng/ml
# 5	250µl of 4	250µl	15.6 ng/ml
# 6	250µl of 5	250µl	7.8 ng/ml
# 7	250µl of 6	250µl	3.9 ng/ml



Detection Antibody - Reconstitute the **Detection Antibody Concentrate** with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

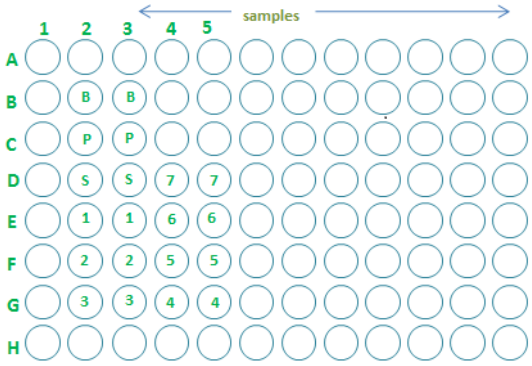
Anti Rabbit IgG-HRP Conjugate - Transfer 120 µL of 100-fold concentrated stock solution to 11.88 mL of **ARIGHRP Diluent Solution (DB08)** to prepare working solution **(protect from light)**. **Note:** 1X working solution of Anti Rabbit IgG-HRP Conjugate should be used within a few days.

Positive Control - Reconstitute the positive control with 1.0 mL of Dilution Buffer to make positive control working solution. **Note:** Positive control working solution should be used within a few days.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack and seal.
3. Add 100 µL of Dilution Buffer to Blank wells (B2, B3).
4. Add 100 µL of Standard (D2, D3 to G2, G3 and G4, G5 to D4, D5), sample, or positive control (C2, C3) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, 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.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Anti Rabbit IgG-HRP Conjugate working solution to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 8-12 minutes at room temperature. **Protect from light.**
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw

a best fit curve through the points on the graph. The data may be linearized by plotting the log of the CALRETICULIN concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CALRETICULIN (NG/ML)	CORRECTED O.D. (450NM)
Blank	0 (0.140)
3.9	0.048
7.8	0.092
15.6	0.141
31.3	0.184
62.5	0.357
125	0.529
250	0.832
500	1.102

- **Lot No.:**
- **Positive Control : 25 - 55 ng/mL**

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human CALRETICULIN.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of CALRETICULIN was 1 ng/mL.

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human Calreticulin	100
Human Fetuin A	0
Human OPG	0
Human OPN	0
Human BMP8B	0
Human BMP5	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard, samples, positive control to each well. Incubate for 2 hours at RT
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate for 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Anti Rabbit IgG-HRP conjugate working solution to each well. Incubate 60 min on plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 8-12 min on the plate shaker. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min