



Human CALML5 ELISA Kit

Enzyme Immunoassay for the quantification of Human CALML5 in Serum, plasma and cell culture supernatants.

Catalog number: ARG82604

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

This gene encodes a novel calcium binding protein expressed in the epidermis and related to the calmodulin family of calcium binding proteins. Functional studies with recombinant protein demonstrate it does bind calcium and undergoes a conformational change when it does so. Abundant expression is detected only in reconstructed epidermis and is restricted to differentiating keratinocytes. In addition, it can associate with transglutaminase 3, shown to be a key enzyme in the terminal differentiation of keratinocytes. [provided by RefSeq, Jul 2008]

Binds calcium. May be involved in terminal differentiation of keratinocytes. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for CALML5 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any CALML5 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for CALML5 is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of CALML5 bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm \pm 2nm. The concentration of

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CALML5 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard (Lyophilized)	2 X 3 ng/Vial	4°C
Standard diluent buffer	5 ml	4°C
Antibody conjugate concentrate	1 vial (120 µl)	4°C
HRP-Streptavidin concentrate	1 vial (150 µl)	4°C (Protect from light)
10X Assay buffer	5 ml	4°C
20X Wash buffer	50 ml	4°C
TMB substrate	15 ml	4°C (Protect from light)
STOP solution	15 ml	4°C
Plate sealer	5 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: for wavelength correction, 570nm or 630nm is recommended)
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- If crystals are observed in the 20X Wash buffer or 10X Assay buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All materials should be equilibrated to room temperature (RT, 22-25°C) 15-20 min before use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Mix the contents of the microplate wells thoroughly by microplate shaker for 30-60 sec or gently tap the plate to ensure good test results. Please mix carefully to avoid well-to-well contamination. Do not reuse microwells.
- The TMB Color developing agent should be colorless and transparent before using.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.

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- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
- Avoid using reagents from different batches.
- Take care not to scratch the inner surface of the microwells.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- It is recommended to add 1X assay buffer, samples, controls and 1X Antibody conjugate into wells within 15 min.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation for 10 min at 300 x g and aliquot & store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1000 x g. Collect serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Collect the

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supernatants and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note:

- a) Samples containing a visible precipitate must be clarified by centrifuge prior to use in the assay.
- b) Do not use haemolytic, icteric or lipaemic specimens.
- c) Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- **1X Wash buffer:** Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 25 ml of 20X Wash buffer + 475 ml of distilled water)
The diluted 1X Wash buffer is stable for 4 weeks at 2°C to 8°C.
- **1X Assay buffer:** Dilute 10X Assay buffer into distilled water to yield 1X Assay buffer. (E.g. 5 ml of 1X Assay buffer + 45 ml of distilled water) The diluted 1X Assay buffer is stable for 4 weeks at 2°C to 8°C.
- **1X Antibody conjugate:** Stock reagent should be mixed well prior to making dilutions. 5-10 minutes before use (freshly prepared is recommended), dilute 100X antibody conjugate concentrate into 1X Assay buffer to yield 1X detection antibody solution, mix well. (e.g. 10 μ l of 100X Antibody conjugate concentrate + 990 μ l of 1X Assay buffer) The diluted antibody solution must be used within 30 minutes after dilution.
- **1X HRP-Streptavidin Solution:** Stock reagent should be mixed well prior to making dilutions. 5-10 minutes before use (freshly prepared is recommended), dilute 100X HRP-Streptavidin concentrate solution into

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1X Assay buffer to yield 1X HRP-Streptavidin Solution buffer, mix well. (e.g. 10 µl of 100X HRP-Streptavidin concentrate solution + 990 µl of 1X Assay buffer) The diluted HRP-Streptavidin Solution must be used within 30 minutes after dilution.

- **Sample:** Plasma or serum sample should be diluted 1:4 with 1 x Assay Buffer (add one part of sample into four parts of 1X assay buffer) and mix well prior to assay (dilution factor = 5). Cell culture supernatant samples may be assayed directly. If the measuring absorbance of samples is higher than the highest standard, dilute the serum or plasma samples with 1X Assay buffer before assay; dilute the cell culture samples with cell culture medium and assay again. For the calculation of the concentrations this dilution factor has to be taken into account.

(It is recommended to do pre-test to determine the suitable dilution factor).

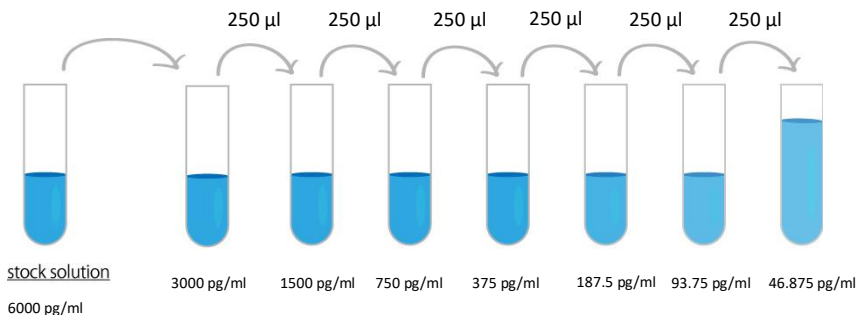
- **Standards:** Reconstitute the standard with 500 µl distilled water to yield a stock concentration of 6000 pg/ml. Allow the stock standard to sit for 10-30 minutes with gentle agitation to make sure the standard is dissolved completely before making serial dilutions. Do not induce foaming.

Standards for serum or plasma samples: Mix 250 µl of the reconstituted standard with 250 µl Standard diluent buffer to yield a stock concentration of 3000 pg/ml. The standard diluent buffer serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: 3000 pg/ml, 1500 pg/ml, 750 pg/ml, 375 pg/ml, 187.5 pg/ml, 93.75 pg/ml, 46.875

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pg/ml.

Standards for cell culture supernatants: Mix 250 μ l of the reconstituted standard with 250 μ l cell culture medium to yield a stock concentration of 3000 pg/ml. The cell culture medium serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: 3000 pg/ml, 1500 pg/ml, 750 pg/ml, 375 pg/ml, 187.5 pg/ml, 93.75 pg/ml, 46.875 pg/ml.



Dilute CALML5 standard as according to the table below:

Standard	CALML5 Conc.	μ l of diluent	μ l of standard
S7	3000 pg/ml	250	250 (6000 pg/ml Stock)
S6	1500 pg/ml	250	250 (S7)
S5	750 pg/ml	250	250 (S6)
S4	375 pg/ml	250	250 (S5)
S3	187.5 pg/ml	250	250 (S4)
S2	93.75 pg/ml	250	250 (S3)
S1	46.875 pg/ml	250	250 (S2)
S0	0	250	0

ASSAY PROCEDURE

All materials including microplate, samples, standards and working solutions should be equilibrated to room temperature (20-25°C, RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Wash the plate **once** by filling each well with **1× Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher, keep the wash buffer in the wells for 30 sec before remove. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. DO NOT let the wells completely dry at any time.
3. Add **100 µl** of **samples standards and zero controls** (Standard diluent buffer or cell culture medium) into appropriate wells.
4. Add **50 µl** of **1X Antibody conjugate** into wells. Cover wells and incubate for 2 hour at RT on a microplate shaker set at 300 rpm.
5. Cover wells and incubate for **2 hour at RT** on a microplate shaker (~300 rpm).
6. Aspirate each well and wash for a **total six washes** as step 2.
7. Add **100 µl** of **1X HRP-Streptavidin solution** to each well. Cover wells and incubate for **45 minutes at RT** on a microplate shaker (~300 rpm).
8. Aspirate each well and wash as step 8 (for a **total six washes**).
9. Add **100 µl** of **TMB Reagent** to each well. Incubate for **5-30 minutes at RT** in dark.

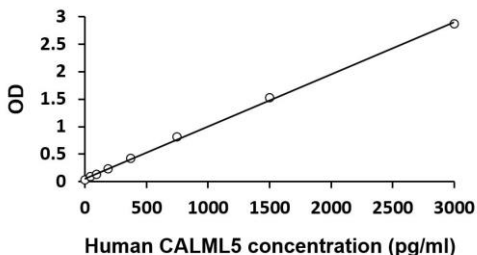
10. Add **100 µl** of **Stop Solution** to each well. The color of the solution should change from blue to yellow.
11. Read the OD with a microplate reader at **450 nm** immediately. (optional: read at 570 nm or 630 nm as the reference wave length) It is recommended read the absorbance within 30 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Human CALML5 ranged from 46.9-3000 pg/ml. The mean MDD was 23.5 pg/ml.

Specificity

This assay recognizes natural and recombinant Human CALML5. No significant cross-reactivity or interference with the factors below was observed:

Human: CALML3, GM-CSF, IFN gamma, IL1 beta, IL2, IL4, IL5, IL6, IL8, IL10, IL12, IL17A, IL18, IL21, IL22, IL23, MCP1, MIP1 beta, SCF, TGF beta 1, TNF alpha and VEGF.

Mouse: GM-CSF, IFN gamma, IL1 beta, IL2, IL4, IL6, IL10, IL17A and TNF alpha.

Rat: IFN gamma, IL1 beta, IL4, IL6, IL10 and TNF alpha.

Recovery

78-120%

Linearity

106-120%

Intra-assay and Inter-assay precision

The CV values of intra-assay was 4.2% and inter-assay was 2%.