Quantikine® ELISA

Human Calbindin D Immunoassay

Catalog Number DCALD0

For the quantitative determination of human Calbindin D concentrations in tissue lysate and urine.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Calbindin D is a 28-30 kDa member of the Vitamin D-dependent calcium-binding proteins family that also includes Parvalbumin, Calmodulin, S100 Protein G, and Troponin C. It contains six EF hand domains of which four are active calcium binding domains (1). Calbindin shows 98% amino acid sequence identity with both mouse and rat Calbindin D. EF hand calcium binding proteins are subdivided into two groups, calcium buffers and calcium sensors (2). Calbindin D is unusual in that it functions as both. As a calcium buffer, Calbindin D is involved in selective reabsorption of Ca^{2+} in the kidney and the intestine as well as regulating the release of insulin in islet cells of the pancreas (3, 4). In the brain, Calbindin D is essential in neural function, altering synaptic interactions in the hippocampus and modulating calcium-channel activity and neuronal firing (3). However, unlike traditional calcium sensors, Calbindin D can bind target proteins such as IMPase or RanBPM in both its apo and Ca^{2+}-loaded states (5, 6). Calbindin D also interacts directly with Caspase-3 and inhibits the apoptosis of neuronal cells, osteocytes, and osteoblasts (7, 8). This interaction is specific among Ca^{2+}-binding proteins and requires conformational changes of Calbindin D induced by its Ca^{2+} binding (9, 10). Calbindin D depletion in mice suggests that it participates in the pathogenesis of Alzheimer's Disease by exacerbating neuronal and synaptic loss, apoptotic cell death, and mitochondrial dysfunction (11). Calbindin D is also expressed in renal distal tubule epithelium, and its levels in the urine can be elevated following kidney damage (12, 13).

The Quantikine Human Calbindin D Immunoassay is a 4.5 hour solid phase ELISA designed to measure human Calbindin D in tissue lysates and urine. It contains *E. coli*-expressed recombinant human Calbindin D and antibodies raised against the recombinant factor. Natural human Calbindin D showed dose-response curves that were parallel to the standard curves obtained using the recombinant Quantikine kit standards. These results indicate that this kit can be used to determine relative levels of natural human Calbindin D.

PRINCIPLE OF THE ASSAY

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

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- 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.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent 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.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Calbindin D Microplate	894773	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Calbindin D.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human Calbindin D Standard	894775	2 vials of recombinant human Calbindin D in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Discard after use. Use a new standard for each assay.
Human Calbindin D Conjugate	894774	21 mL of a polyclonal antibody specific for human Calbindin D conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895117	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-10	895266	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Test tubes for dilution of standards and samples.
- Human Calbindin D Controls (optional; R&D Systems, Catalog # QC189).

Supplies required for tissue lysate samples:

- RIPA buffer plus protease inhibitors

SAMPLE COLLECTION & STORAGE

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

Tissue Lysates - Tissue must be lysed prior to assay as directed in the Sample Values section.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter. Assay immediately, or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Tissue lysate samples require at least a 10-fold dilution. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Calibrator Diluent RD5-10.

Urine samples require a 2-fold dilution. A suggested 2-fold dilution is 200 µL of sample + 200 µL of Calibrator Diluent RD5-10.

REAGENT PREPARATION

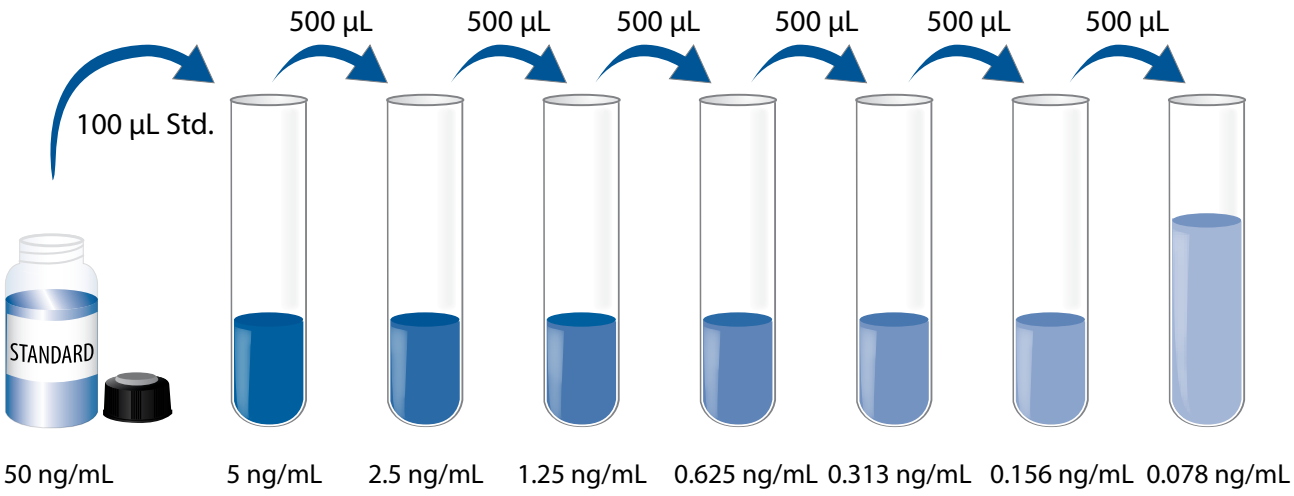
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Human Calbindin D Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Calbindin D Standard with deionized or distilled water. This reconstitution produces a stock solution of 50 ng/mL. Mix the standard to ensure complete reconstitution, and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-10 into the 5 ng/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 5 ng/mL standard serves as the high standard. Calibrator Diluent RD5-10 serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

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

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1W to each well.
4. Add 50 µL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 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 200 µL of Human Calbindin D Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50 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 the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

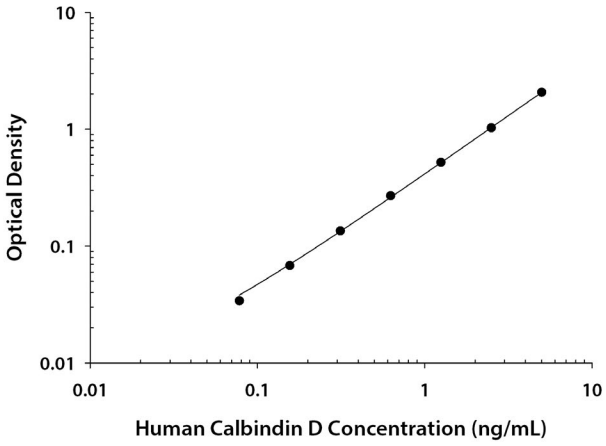
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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 human Calbindin D 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.

Since 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.



(ng/mL)	O.D.	Average	Corrected
0	0.018 0.019	0.019	—
0.078	0.052 0.054	0.053	0.034
0.156	0.086 0.088	0.087	0.068
0.313	0.151 0.156	0.154	0.135
0.625	0.281 0.295	0.288	0.269
1.25	0.532 0.545	0.539	0.520
2.5	1.011 1.081	1.046	1.027
5	2.036 2.145	2.091	2.072

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/mL)	0.511	1.65	2.84	0.548	1.57	2.96
Standard deviation	0.026	0.040	0.072	0.038	0.075	0.150
CV (%)	5.1	2.4	2.5	6.9	4.8	5.1

RECOVERY

The recovery of human Calbindin D spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Urine (n=4)	93	89-98%

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of human Calbindin D were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay.

		Tissue lysate (n=1)	Urine (n=4)
1:2	Average % of Expected	91	100
	Range (%)	———	96-102
1:4	Average % of Expected	86	95
	Range (%)	———	90-99
1:8	Average % of Expected	81	92
	Range (%)	———	83-100
1:16	Average % of Expected	82	88
	Range (%)	———	82-96

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of human Calbindin D ranged from 0.003-0.015 ng/mL. The mean MDD was 0.007 ng/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human Calbindin D produced at R&D Systems.

SAMPLE VALUES

Urine - Samples from apparently healthy volunteers were evaluated for the presence of human Calbindin D in this assay. Data was normalized to creatinine. No medical histories were available for the donors used in this study.

Sample Type	Mean (µg/g creatinine)	Range (µg/g creatinine)	Standard Deviation (µg/g creatinine)
Urine (n=23)	1.70	0.395-6.19	1.69

Tissue Lysates - Human kidney tissue was placed in cold RIPA buffer plus protease inhibitors and homogenized on ice with a polytron for 10-15 seconds. Tissue was then centrifuged to remove debris. An aliquot of the tissue was removed, assayed for human Calbindin D, and measured 424 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human Calbindin D.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human Calbindin D control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Calmodulin
Parvalbumin
S100A1
S100A2
S100A4
S100A6
S100A7
S100A8
S100A8/9
S100A9
S100A10
S100A11
S100A13
S100A16
S100B
S100P

Other factors:

Osteocalcin

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