

ab289835 – Rat Calcitonin ELISA Kit

For the quantitative measurement of Calcitonin in rat serum, plasma and other biological fluids.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<https://www.abcam.com/ab289835>

Storage and Stability

The entire kit may be stored at 4°C for up to 1 months from the date of shipment. After this the components should be stored separately according to the following conditions.

Materials Supplied

Item	Quantity	Storage Condition
Micro ELISA Plate	8 x 12 strips	-20°C
Standard	2 vials	-20°C
Standard/Sample dilution buffer	20 ml	4°C
Biotinylated detection antibody (Concentrated)	120 µl	-20°C
Biotinylated detection antibody diluent	14 ml	4°C
HRP- Conjugate (Avoid light)	120 µl	-20°C
HRP Conjugate diluent	14 ml	4°C
Substrate reagent (Avoid light)	10 ml	4°C
Stop Solution	10 ml	4°C
Wash buffer	30 ml	4°C
Plate sealers	4	RT

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader
- Precision pipettes with disposable tips
- Clean Eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper.
- PBS.

Reagent Preparation

- Prepare reagents within 30 minutes before the experiment. Before using the kit, spin tubes and bring down all components to the bottom of the tubes.

Biotinylated detection antibody working solution: Calculate the required amount before the experiment (100 µl/well). Centrifuge the stock tube before use, dilute the 100x Concentrated Biotinylated Detection Ab to 1x working solution with Biotinylated Detection Ab Diluent

Wash Buffer: Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer.

Δ Note: if crystals have formed in the concentrate, warm it in a 40°C water bath and mix it gently until the crystals have completely dissolved.

HRP- Conjugate: Calculate the required amount before the experiment (100µl/well). Dilute the 100x Concentrated HRP Conjugate to 1x working solution with Concentrated HRP Conjugate Diluent.

Standard Preparation

1. Centrifuge the standard at 10,000×g for 1 min.
2. Add 1.0 mL of Standard & Sample Diluent, let it stand for 10 min and invert it gently several times.
3. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 1000 pg/ml.
4. Then make serial dilutions as needed. The recommended dilution gradient is: 1000, 500, 250, 125, 62.5, 31.25, 15.63, and 0 pg/ml.
5. Prepare 7 tubes, add 500 µl of Standard & Sample Diluent to each tube. Pipette 500 µl of the 1000 pg/ml stock solution to the first tube and mix up to produce a 500 pg/ml working solution. Transfer 500 µl of the solution into the other tube to form 2- fold serial dilutions of the highest standards to make the standard curve within the range of this assay.

Sample Preparation

Δ Note: Samples should be assayed within 7 days when stored at 4°C, otherwise aliquot and stored at -20°C (≤1 month) or -80°C (≤3 months). Avoid repeated freeze-thaw cycles

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 min at 1000×g at 2~8°C. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable and endotoxin free.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000x g at 2~8°C within 30 min of collection. Collect the supernatant to carry out the assay.

Δ Note: Hemolysed samples are not suitable for ELISA assay!

Cell lysates: For adherent cells, gently wash the cells with moderate amount of pre-cooled PBS and dissociate the cells using trypsin. Collect the cell suspension into a centrifuge tube and centrifuge for 5 min at 1000×g. Discard the medium and wash the cells 3 times with pre-cooled PBS. For each 1×10⁶ cells, add 150-250 µL of pre-cooled PBS to keep the cells suspended. Repeat the freeze-thaw process several times until the cells are fully lysed. Centrifuge for 10 min at 1500×g at 4°C. Remove the cell fragments, collect the supernatant for assay. Avoid repeated freeze-thaw cycles.

Tissue homogenates: It is recommended to get detailed references from the literature before analyzing different tissue types. For general information, hemolysed blood may affect the results, so the tissues should be minced into small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 min at 5000xg to get the supernatant.

Cell culture supernatant or other biological fluids: Centrifuge samples for 20 min at 1000×g at 2- 8 oC. Collect the supernatant for assay.

End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration falls in the optimal detection range of the kit.

Assay Protocol

Δ Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1. Add 100 µl of each standard or samples into appropriate wells. Cover the plate with the plate sealer provided in the kit and incubate for 90 min at 37°C.
Δ Note: solutions should be added to the bottom of the micro-ELISA plate well, avoid touching the inside wall and bubble formation as much as possible.
2. Remove the liquid out of each well. Do not wash. Immediately add 100 µl of Biotinylated Detection Antibody working solution to each well. Cover the plate with the sealer provided in the kit. Gently mix and incubate for 1 hr. at 37°C.
3. Aspirate the solution from each well add 350 µl of 1x wash buffer to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times.
Δ Note: a microplate washer can be used in this step and other wash steps.
4. Add 100 µl of HRP Conjugate working solution to each well. Cover with the Plate sealer. Incubate for 30 min at 37°C.
5. Aspirate the solution from each well, repeat the wash process for five times as conducted in step 3.
6. Add 90 µl of Substrate Reagent to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min.
7. Add 50 µl of Stop Solution to each well. Note: adding the stop solution should be done in the same order as the substrate solution
8. Read the absorbance in micro plate reader set to 450 nm.

Calculation:

- Determine the average of the duplicate readings for each standard and samples then subtract the average zero standard optical density.
- Plot a four-parameter logistic with standard concentration on the x-axis and OD values on the y-axis.
- If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.
- If the OD of the sample is under the lowest limit of the standard curve, retest the samples with appropriate dilution.
- The actual concentration is the concentration obtained by calculated multiplied by the dilution factor.

Recovery

Matrix	Recovery Range (%)	Average (%)
Serum (n=5)	92-105	99
EDTA plasma (n=5)	86-98	93

Linearity

Sample	1:2	1:4	1:8	1:16
Serum (n=5)	92-110%	98-116%	101-116%	95-109%
EDTA plasma (n=5)	90-104%	86-100%	88-89%	85-98%

Technical Support

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