Human Calcitonin Gene Related Peptide ELISA Kit (CGRP)

Catalog Number: RK01104

This ELISA kit used for quantitative determination of CGRP in human serum, plasma, tissue homogenates, cell lysates, cell culture supernates and other biological fluids. For research use only, and it's highly recommended to read thoroughly of this manual before using the product.

Manufactured by

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Introduction

The kit is a competitive inhibition enzyme immunoassay technique for the in vitro quantitative measurement of CGRP in human serum, plasma, tissue homogenates, cell lysates, cell culture supernates and other biological fluids.

Principle of The Assay

This assay employs the competitive inhibition enzyme immunoassay technique. An antibody specific to CGRP has been pre-coated onto a microplate. A competitive inhibition reaction is launched between biotin labeled growth hormone and unlabeled CGRP (Standards or samples) with the pre-coated antibody specific to CGRP. After incubation the unbound conjugate is washed off. Next, avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. The amount of bound HRP conjugate is reverse proportional to the concentration of CGRP in the sample. After addition of the substrate solution, the intensity of color developed is reverse proportional to the concentration of CGRP in the sample.

Materials Provided

Part	Size (96T)	Cat NO.	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Antibody Coated Plate	**8×12**	**RM06517**	Return unused wells to the foil pouch containing the desiccant pack and store at ≤ -20 °C. Reseal along entire edge of zip-seal.
Standard Lyophilized	**2**	**RM06518**	Aliquot and store at ≤ -20 °C in a manual defrost freezer. * Avoid repeated freeze-thaw cycles.
Concentrated Biotin Conjugate Antigen(100×)	**1 ×120ul**	**RM06519**	May be stored for up to 6 month at 2-8 °C.*
Streptavidin-HRP Concentrated (100×)	**1 ×120ul**	**RM06520**	
Standard/Sample Diluent (R1)	**1 ×20mL**	**RM00023**	May be stored for up to 6 month at 2-8 °C.*
Biotin Conjugate Antigen Diluent (R2)	**1 ×12mL**	**RM00024**	
Streptavidin-HRP Diluent(R3)	**1 ×12mL**	**RM00025**	
Wash Buffer(30x)	**1 × 20mL**	**RM00026**	
TMB Substrate	**1 ×9 mL**	**RM00027**	
Stop Solution	**1 ×6 mL**	**RM00028**	
Plate Sealers	**4 strips**		
Specification	**1**		

Sample Collection and Storage

1. **Cell Culture Supernates:**

   Centrifuge 1000x g for 10 min and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze/thaw cycles. If cell culture supernate samples require larger dilutions, perform an intermediate dilution with culture media and the final dilution with the Standard/Sample Diluent(R1).

2. **Serum:**

   Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1,000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

3. **Plasma:**

   Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000×g at 2-8°C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

4. **Cell Lysates:**

   Cells need to be lysed before assaying according to the following directions. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1,000 × g for 5 minutes (suspension cells can be collected by centrifugation directly). Wash cells three times in cold PBS. Resuspend cells in fresh lysis buffer with concentration of 10^7 cells/ml. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified. Centrifuge at 1,500 × g for 10

minutes at 2-8°C to remove cellular debris. Assay immediately or aliquot and store at ≤-20°C.

5. **Tissue homogenates**

   The preparation of tissue homogenates will vary depending upon tissue type. Tissues were rinsed in ice-cold PBS to remove excess blood thoroughly and weighed before homogenization. Minced the tissues to small pieces and homogenized them in fresh lysis buffer (different lysis buffer needs to be chosen based on subcellular location of the target protein) (e.g. 1mL lysis buffer is added in 200mg tissue sample) with a glass homogenizer on ice (Micro Tissue Grinders woks, too). The resulting suspension was sonicated with an ultrasonic cell disrupter till the solution is clarified. Then, the homogenates were centrifuged for 5 minutes at 10,000 × g. Collect the supernates and assay immediately or aliquot and store at ≤-20°C.

6. **Other biological fluids**

   Centrifuge samples for 20 minutes at 1,000×g. Collect the supernates and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

7. **Avoid hemolytic and hyperlipidemia sample for Serum and Plasma.**

8. **Dilution:**

   Dilute samples at the appropriate multiple (recommend to do pre-test to determine the dilution factor).

Precautions

1. **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
2. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

3. Variations in sample collection, processing, and storage may cause sample value differences.
4. Reagents may be harmful, if ingested, rinse it with an excess amount of tap water.
5. Stop Solution contains strong acid. Wear eye, hand, and face protection.
6. Apart from the standard of kits, other components should not be refrigerated.
7. Please perform simple centrifugation to collect the liquid before use.
8. Do not mix or substitute reagents with those from other lots or other sources.
9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency.
10. Mix the sample and all components in the kits adequately, and use clean plastic container to prepare all of the diluent.
11. Both the sample and standard should be assayed in duplicate, and the sequence of the regents should be added consistently.
12. Reuse of dissolved standard is not recommended.
13. The kit should not be used beyond the expiration date on the kit label.
14. The kit should be away from light when it is stored or incubated.
15. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
16. To avoid cross contamination, please use disposable pipette tips.
17. Please prepare all the kit components according to the Specification. If the kits will be used several times, please seal the rest strips and preserve with desiccants. Do use up within 2 months.
18. The 48T kit is also suitable for the specification.

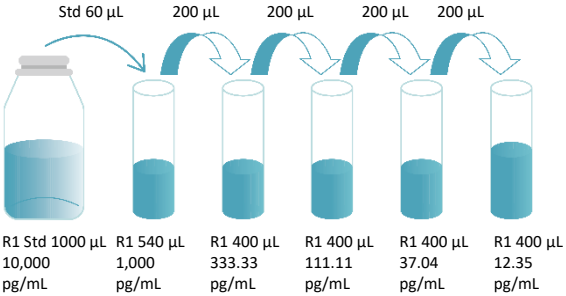
Experiment Materials

1. Microplate reader (measuring absorbance at 450 nm, with the correction wavelength set at 570 nm or 630 nm).
2. Pipettes and pipette tips：0.5-10, 2-20, 20-200, 200-1000 µL.
3. Microplate washer, Squirt bottle.
4. Micro-oscillator.
5. Deionized or double distilled water, graduated cylinder.
6. Polypropylene Test tubes for dilution.
7. Incubator.

Reagent Preparation

1. **Bring all reagents to room temperature before use. If crystals have formed in the concentrate, Bring the reagent to room temperature and mix gently until the crystals have completely dissolved.**

2. **Standard:** Add Standard/Sample Diluent(R1) 1.0mL into freeze-dried standard, sit for a minimum of 15 minutes with gentle agitation prior to making dilutions (10,000pg/mL), Prepare EP tubes containing Standard/Sample Diluent(R1), and produce a dilution series according to the picture shown below (recommended concentration for standard curve: 1,000pg/mL, 333.33pg/mL, 111.11pg/mL, 37.04 pg/mL, 12.35pg/mL). Redissolved standard solution (10,000pg/mL), aliquot and store at -20°C— -70°C.



3. **Concentrated Biotin Conjugate Antigen (100x)**: Dilute to the working concentration with Biotin Conjugate Antigen Diluent (R2).

Dilution Method

Strip	Concentrated Biotin Conjugate Antigen (100x)	Biotin Conjugate Antigen Diluent (R2)
2	20ul	1980ul
4	40ul	3960ul
6	60ul	5940ul
8	80ul	7920ul
10	100ul	9900ul
12	120ul	11880ul

4. **Streptavidin-HRP Concentrated (100x):** Dilute 1:100 with the Streptavidin-HRP Diluent(R3) before use, and the diluted solution should be used within 30 min.

Dilution Method

Strip	Concentrated Streptavidin-HRP (100x)	Streptavidin-HRP Diluent(R3)
2	20ul	1980ul
4	40ul	3960ul
6	60ul	5940ul
8	80ul	7920ul
10	100ul	9900ul
12	120ul	11880ul

5. **Wash buffer**: Dilute 1:30 with double distilled or deionized water before use.

Wash Method

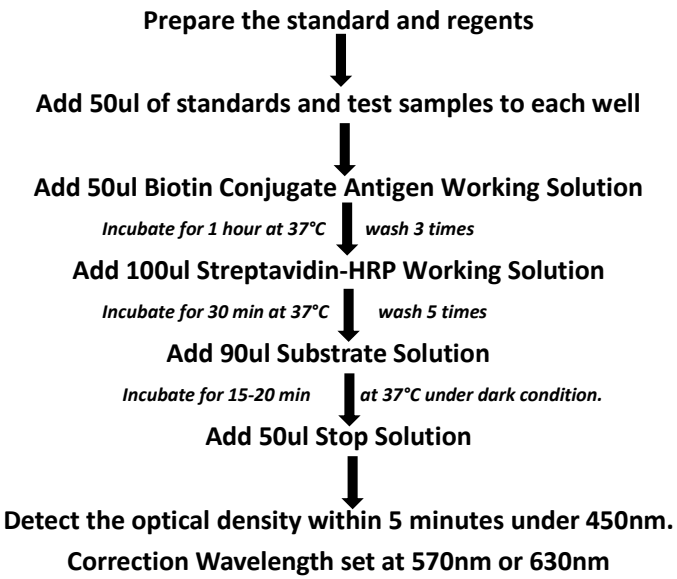
Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with **Wash Buffer**(300ul) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining **Wash Buffer** by aspirating or decanting. Invert the plate and blot it against clean paper towels.

Assay Procedure

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
2. Prepare the Biotin Conjugate Antigen Concentrated (100X) Working Solution 15 minutes early before use.
3. Add 50 µL Standard/sample Diluent (R1) in blank well.
4. Add 50 µL different concentration of standard and sample in other wells, Add then add Biotin Conjugate Antigen Working Solution in other wells (50µL/well), cover with new adhesive strip provided. Incubate for 1 hour at 37°C.
5. Prepare the Streptavidin-HRP Concentrated (100X) Working Solution 15minutes early before use.
6. Remove the liquid of each well, Add wash buffer 350 µL/well, aspirate each well after holding 60-120 seconds, repeating the process two times for a total of three washes.
7. Add Streptavidin-HRP Diluent(R3) in blank well and add Streptavidin-HRP Working Solution in other wells (100 µL/well), cover with new adhesive strip provided. Incubate for 30 minutes at 37°C.
8. Warm-up the Microplate reader.
9. Remove the liquid of each well, Add wash buffer 350 µL/well, aspirate each well after holding 60-120 seconds, repeating the process four times for a total of five washes.
10. Add TMB Substrate (90µL/well). Incubate for 15-20 minutes at 37°C .Protect from light.
11. Add Stop Solution (50µL/well), determine the optical density of each well within 5 minutes, using a Microplate reader set to 450 nm. If wavelength

correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 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.

Assay Procedure Summary



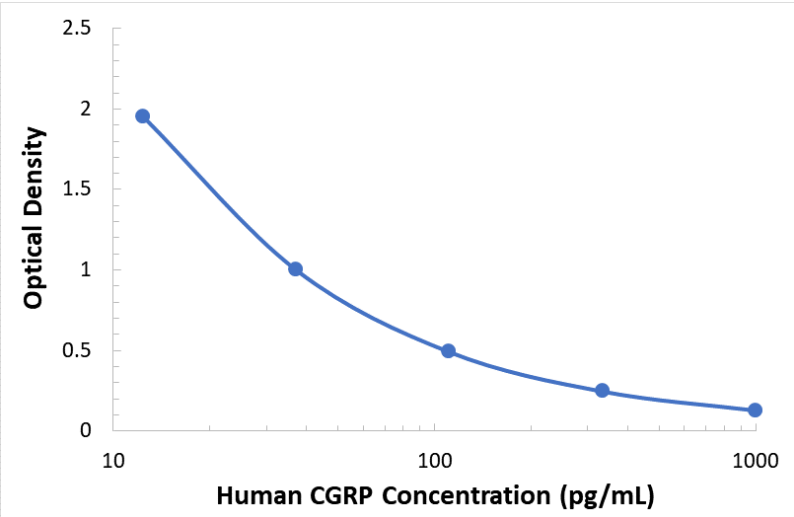
Calculation of Results

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

2. 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 a log/log graph. The data may be linearized by plotting the log of the CGRP concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

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

Typical Data



The standard curves are provided for demonstration only. A standard curve should be generated for each set of CGRP assayed.

Sensitivity

The minimum detectable dose (MDD) of CGRP is typically less than 5.35 pg/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.

Specificity

This assay has high sensitivity and excellent specificity for detection of CGRP.
No significant cross-reactivity or interference between CGRP and analogues was observed.
Note:
Limited by current skills and knowledge, it is impossible for us to complete the cross- reactivity detection between CGRP and all the analogues, therefore, cross reaction may still exist.

Precision

Intra-plate Precision

3 samples with low, middle and high level CGRP were tested 20 times on one plate, respectively.
Intra-Assay: CV<10%

Inter-plate Precision

3 samples with low, middle and high level CGRP were tested on 3 different plates, 8 replicates in each plate.
Inter-Assay: CV<12%

Recovery

Matrices listed below were spiked with certain level of CGRP and the recovery rates were calculated by comparing the measured value to the expected amount of CGRP in samples.

Matrix	Recovery range (%)	Average (%)
serum(n=5)	84-97	92
EDTA plasma(n=5)	80-94	87
heparin plasma(n=5)	81-96	90

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of CGRP and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8	1:16
serum(n=5)	90-99%	79-98%	86-101%	80-96 %
EDTA plasma(n=5)	82-97%	89-105%	78-91%	83-97%
heparin plasma(n=5)	93-102%	81-94%	84-104%	85-101 %