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CGRP antibody detection kit

Catalog #: CGRP-ELISA-5 Lot #: FGI-1003.3

Product Description Sheet

Product description: Enzyme Linked Immunosorbent Assay (ELISA) based CGRP immune response detection kit. The kit is designed to detect the presence of anti-human CGRP antibodies in serum samples. The high binding capacity ELISA plates are precoated with CGRP antigen and blocked for ready-to-use application. The CGRP antibodies specifically bind to plates, after removal of non-specific interaction of proteins the presence of anti-CGRP antibodies are detected by Alkaline phosphatase-conjugated secondary antibodies. The presence of anti-human CGRP antibodies are quantitated by colorimetric determination. The kit comes with positive and negative controls for mouse and rabbit secondary antibodies.

Kit contents:

Kit contains sufficient reagents to process 5 96 well ELISA plates

Description	Catalog #	Quantity
• Antigen pre-coated and blocked 96 well plates	FGI-1960	2 Plates
• Positive control antibody	CGRP-112AP	50 ul
• Wash buffer	FGI-1961	5000 ml
• Antibody Dilution buffer	FGI 1963	50 ml
• Secondary Antibody Conjugate	FGI-1982	100 ul
• Alkaline Phosphatase substrate buffer (5X)	FGI-1964	10 ml
• Alkaline Phosphatase substrate tablets.	FGI-1965	8 Tabs
• Stopping Buffer	FGI-1966	15 ml

Reagents/instruments required for running the test:

- 10ul. 100ul and 200 ul pipettes
- ELISA reader
- ELISA plate washer (not mandatory)
- Humidified chambers
- Orbital shaker

Introduction:

In order to test the effectiveness of immunization, the pre-immune serum and the test bleeds are monitored for the presence of antibodies against a given antigen. The test is carried out using Enzyme Linked Immuno-Sorbent Assay (ELISA). The test is based on the binding of CGRP

antibodies to the immobilized antigen on ELISA plate. The excess antibodies are removed by washing. The presence of bound antibodies is detected by conjugated secondary antibodies and chromogenic substrates. After optimum color development the enzymatic reaction is stopped by addition of stop solution and the optical density at 405 nm is measured in ELISA plate reader.

Procedure:

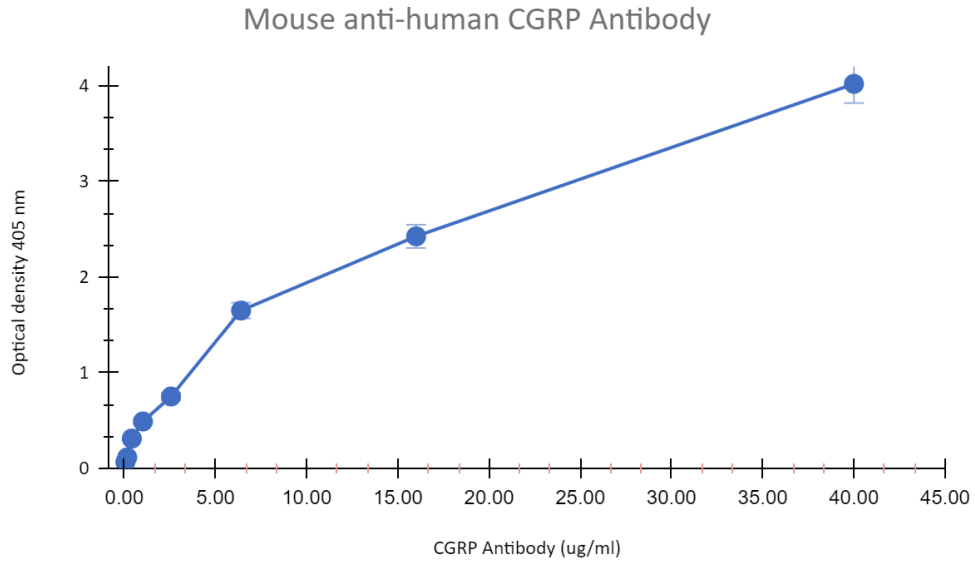
In order to get consistent and reproducible results using CGRP antibody detection kit the following steps need to be performed.

In the supplied 96 well ELISA plate, the samples and standards are added as shown in the diagram below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
B	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
C												
D												
E												
F												
G												
H	Std1	Std2	Std3	Std4	Std5		PI1	PI2	PI3	PI4	Blank	Blank

1. Dilute sample S1 through S12 in PBS or antibody dilution buffer and add 100ul of sample in duplicate to respective wells (A1-A12 and B1-B12). More dilutions or more samples can be applied to remaining wells on row C-G. It is recommended to perform assay in duplicate for each sample. To determine the titers, each sample can be serially diluted and applied in wells A1-G1. To compare immune vs preimmune serum, dilute the sample appropriately and add 100 ul samples in adjacent wells in duplicate.
2. Apply 100ul of Standards (Std1 through Std5) in wells H1-H5.
3. If pre-immune serum samples are available, use 100ul of appropriately diluted samples in well H7 through H10.
4. Wells H6, H11 and H12 will be left blank.
5. Cover and incubate plates in humidified chambers at room temperature for 60 minutes.
6. Aspirate the liquid from each well and tap dry the plate on a stack of filter paper to remove residual antibody from each well.
7. Wash each well with 250ul of wash buffer, incubated for 5 minutes with shaking. Remove and discard solution, tap dry the wells. Repeat wash steps 2 more times. Note: Do not allow the plates to go dry, if next steps are not performed immediately, keep the plates in humidified chambers before moving to the next step.
8. Freshly dilute (1:3000 v/v) the secondary antibody in antibody dilution. For each plate make 6ml of secondary antibody solution (2ul of antibody to each 6ml of antibody dilution buffer).
9. Pipette 50 ul of secondary antibody in all wells and incubate in a humidified chamber at room temperature for 1 hour.
10. Aspirate secondary antibody and tap dry ELISA plate. Wash all wells with 250 ul of wash buffer. Incubate with shaking for 5 minutes. Aspirate and tap-dry the plate. Repeat the wash step 2 more times.

11. Freshly prepare 1X substrate buffer by diluting 2ml into 8 ml of distilled water, add 2 chromogenic substrate tablets in 10 ml of 1X substrate buffer. Add 100ul of substrate in to each well. Discard the remaining solution. *Note:* the concentration of substrate buffer can be reduced 10-fold depending upon the buffering capacity desired. We recommend that are obtained. *Note: Avoid touching the substrate tablets with hands.*
12. Add 100ul of substrate to each well and add 2ul of secondary diluted antibody to well H6, incubate for up to 30 minutes until sufficient color is developed..
13. To stop the reaction, add 50ul of stop solution to each well.
14. Read the absorbance at 405 nm, alternatively, the absorbance can be monitored in a kinetic ELISA reader. Antibody titer is expressed as a reciprocal of the serum dilution that results in twice the optical density obtained by pre-immune or normal serum at similar dilution.



General Recommendations:

Optimization of these reagents for your application may be necessary only if the standard protocol gives high background or false positive results. If high back ground is obtained, the following steps are recommended:

1. Try avoiding touching or scratching the wells with pipette tips at all times. Use clean plastic and glassware to avoid contamination.
2. Optimize the washing steps by increasing the number of washes, wash buffer volume and incubation time. Additionally, the detergent (Triton X`100 at concentration 0,1% can be added to the wash buffer if there is a high background in H6 well.
3. Increase the dilution of primary and secondary antibody
4. Reduce the concentration of substrate buffer from 1X to 0.75 X or 0.5 X
5. Reduce the incubation time with substrate solution.

6. Include a positive control (by incubating 2ul of secondary antibody solution with 100ul of substrate solution), Negative control (by omitting the secondary antibody) and a reagent blank (by omitting the primary antibody or by adding denatured secondary antibody).