3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Neurokinin A EIA Kit
for serum, plasma, culture supernatant, and cell lysates

Catalog Number **RAB0455**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The Neurokinin A Enzyme Immunoassay (EIA) Kit is an *in vitro* quantitative assay for detecting Neurokinin A peptide based on the principle of competitive enzyme immunoassay. The microplate in the kit is pre-coated with anti-rabbit secondary antibody. After a blocking step and incubation of the plate with anti-Neurokinin A antibody, both biotinylated Neurokinin A peptide, and peptide standard or targeted peptide in samples interacts competitively with the Neurokinin A antibody. Uncompeted (bound) biotinylated Neurokinin A peptide then interacts with Streptavidin-horseradish peroxidase (SA-HRP) which catalyzes a color development reaction. The intensity of colorimetric signal is directly proportional to the amount of biotinylated peptide-SA-HRP complex and inversely proportional to the amount of Neurokinin A peptide in the standard or samples. This is due to the competitive binding to Neurokinin A antibody between biotinylated Neurokinin A peptide and peptides in standard or samples. A standard curve of known concentration of Neurokinin A peptide can be established and the concentration of Neurokinin A peptide in the samples can be calculated accordingly.

Components

1. 96-well plate coated with secondary antibody (Item A) - RAB0455A: 96 wells (12 strips × 8 wells) coated with secondary antibody.
2. 20x Wash Buffer (Item B) - RABWASH3: 25 mL
3. EIA Neurokinin-A Peptide standard (Item C) - RAB0455C: 2 vials, 10 µL/vial.
4. Anti-Neurokinin A Detection Antibody (Item N) - RAB0455F: 2 vials, 5 µL/vial.
5. EIA Assay Diluent A (Item D) - RABDIL9: 30 mL, contains 0.09% sodium azide as preservative. Diluent for standards and serum or plasma samples.
6. EIA 5x Assay Diluent B (Item E) - RABDIL10: 15 mL of 5x concentrated buffer. Diluent for standards and cell culture media or other sample types.
7. Biotinylated Neurokinin A Peptide (Item F) - RAB0455G: 2 vials, 20 µL/vial.
8. HRP-streptavidin (Item G) - RABHRP3: 600 µL of 160x concentrated HRP-conjugated Streptavidin.
9. Neurokinin A Positive Control Sample (Item M) - RAB0455K: 1 vial, 100 µL.
10. TMB Substrate solution (Item H) - RABTMB2: 12 mL of 3,3′,5,5′- tetramethylbenzidine (TMB) in buffered solution.
11. Stop Solution (Item I) – RABSTOP3: 8 mL of 0.2 M sulfuric acid.

Reagents and Equipment Required but Not Provided.

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µL to 1 mL volumes.
3. Adjustable 1-25 mL pipettes for reagent preparation.
4. 100 mL and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. SigmaPlot software (or other software which can perform four-parameter logistic regression models).
8. Tubes to prepare standard or sample dilutions.
9. Orbital shaker.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices

Preparation Instructions

If testing plasma or serum samples, use Assay Diluent A to dilute Item F and Item C. If testing cell culture media or other sample types, use Assay Diluent B to dilute Item F and Item C. For sample and positive control dilutions, refer to steps 6, 7, 8, and 10 of Preparation.

1. Keep kit reagents on ice during reagent preparation steps. Equilibrate plate to room temperature before opening the sealed pouch.

2. Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water.

3. Briefly centrifuge the Anti-Neurokinin A Antibody vial (Item N) before use. Add 50 µL of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently.

4. The antibody concentrate should then be diluted 100-fold with 1x Assay Diluent B. This is the anti-Neurokinin A antibody working solution, which will be used in Procedure, step 2.

Note: The following steps may be done during the antibody incubation procedure (Procedure, step 2).

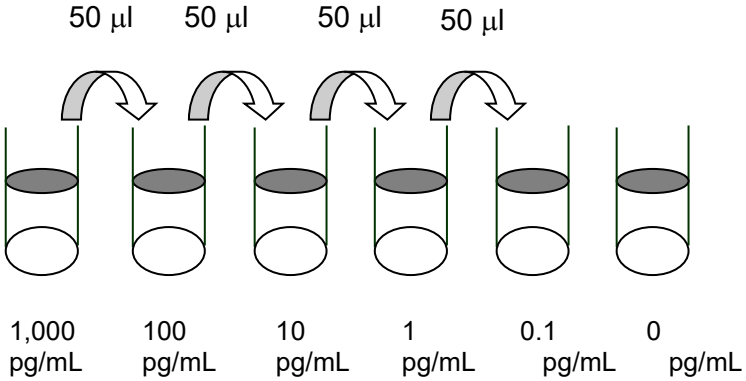
5. Briefly centrifuge the vial of Biotinylated Neurokinin A (Item F) before use. Add 5 µL of Item F to 5 mL of the appropriate Assay Diluent. Pipette up and down to mix gently. The final concentration of biotinylated Neurokinin A will be 10 pg/mL. This solution will only be used as the diluent in Preparation, step 6.

6. Preparation of Standards: Label 6 microtubes with the following concentrations: 1,000 pg/mL, 100 pg/mL, 10 pg/mL, 1 pg/mL, 0.1 pg/mL and 0 pg/mL. Pipette 450 µL of biotinylated Neurokinin A solution into each tube, except for the 1,000 pg/mL (leave this one empty).

   Note: It is very important to make sure the concentration of biotinylated Neurokinin A is 10 pg/mL in all standards.

   a. Briefly centrifuge the vial of Neurokinin A (Item C). In the tube labeled 1,000 pg/mL, pipette 8 µL of Item C and 792 µL of 10 pg/mL biotinylated Neurokinin A solution (Preparation, step 5). This is the Neurokinin A stock solution (1,000 pg/mL Neurokinin A and 10 pg/mL biotinylated Neurokinin A). Mix thoroughly. This solution serves as the first standard.
   b. To make the 100 pg/mL standard, pipette 50 µL of Neurokinin A stock solution the tube labeled 100 pg/mL. Mix thoroughly.
   c. Repeat this step with each successive concentration, preparing a dilution series (see Figure 1). Each time, use 450 µL of biotinylated Neurokinin A and 50 µL of the prior concentration until 0.1 pg/mL is reached. Mix each tube thoroughly before the next transfer.
   d. The final tube (0 pg/mL Neurokinin A and 10 pg/mL biotinylated Neurokinin A) serves as the zero standard (or total binding).

Figure 1.
Dilution Series for Standards



7. Prepare a 10-fold dilution of Item F. To do this, add 2 µL of Item F to 18 µL of the appropriate Assay Diluent. This solution will be used in steps 8 and 10.

8. Positive Control Preparation: briefly centrifuge the positive control vial (Item M). To the tube of Item M, add 101 µL of 1x Assay Diluent B. Also add 2 µL of 10-fold diluted Item F (Preparation, step 7) to the tube. This is a 2-fold dilution of the positive control. Mix thoroughly. The positive control is a cell culture medium sample with an expected signal between 10–30% of total binding (70–90% of competition) if diluted as described. It may be diluted further if desired, but be sure the final concentration of biotinylated Neurokinin A is 10 pg/mL.

9. If Item B (20x Wash Concentrate) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1x Wash Buffer.

10. Sample Preparation: Use Assay Diluent A plus biotinylated Neurokinin A to dilute serum/plasma samples. For cell culture medium and other sample types, use 1x Assay Diluent B plus biotinylated Neurokinin A as the diluent.

    Note: It is very important to make sure the final concentration of the biotinylated Neurokinin A is 10 pg/mL in every sample.

    For example, to make a 4-fold dilution of sample, mix together 2.5 µL of 10-fold diluted Item F (Preparation, step 7), 185 µL of appropriate Assay Diluent, and 62.5 µL of the sample; mix gently. The total volume is 250 µL, enough for duplicate wells on the microplate.

    Do not use Item F diluent from Preparation, step 5 for sample preparation.

    If undiluted samples are used, biotinylated Neurokinin A must be added to a final concentration of 10 pg/mL. For example, add 2.5 µL of 10-fold diluted Item F to 247.5 µL of sample.

11. Briefly centrifuge the HRP-Streptavidin vial (Item G) before use. The HRP-Streptavidin concentrate should be diluted 160-fold with 1x Assay Diluent B.

    Note: Do not use Assay Diluent A for HRP-Streptavidin from Preparation, step 10.

Storage/Stability

Standard Neurokinin A peptide, Biotinylated Neurokinin A peptide, and Positive Control should be stored at –20 °C or –70 °C (recommended at –70 °C) after arrival. Avoid repeated freeze-thaw cycles.

The remaining kit components may be stored at –20 °C.

Opened microplate strips and Item N may be store for up to 1 month at 2–8 °C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

The kit remains active for up to 1 year.

Procedure

1. Keep kit reagents on ice during reagent preparation steps. It is recommended that all standards and samples be run at least in duplicate.

2. Add 100 µL of anti-Neurokinin A antibody (see Preparation, step 4) to each well. Incubate for 1.5 hours at room temperature with gentle shaking (1–2 cycles/sec) or incubate overnight at 4 °C.

3. Discard the solution and wash wells 4 times with 1x Wash Buffer (200–300 µL each). Washing may be done with a multichannel pipette or an automated plate washer. Complete removal of liquid at each step is essential to good assay performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 100 µL of each standard (see Preparation, step 6), positive control (see Preparation, step 8), and sample (see Preparation, step 10) into appropriate wells. Be sure to include a blank well (Assay Diluent only). Cover wells and incubate for 2.5 hours at room temperature with gentle shaking (1–2 cycles/sec) or overnight at 4 °C.

5. Discard the solution and wash 4 times as directed in step 3.

6. Add 100 µL of prepared HRP-Streptavidin solution (see Preparation, step 11) to each well. Incubate for 45 minutes with gentle shaking at room temperature. It is recommended that incubation time should not be shorter or longer than 45 minutes.

7. Discard the solution and wash 4 times as directed in step 3.

8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking (1–2 cycles/sec).

9. Add 50 µL of Stop Solution (Item I) to each well. Read absorbances at 450 nm immediately.

Results

Calculations

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the blank optical density. Plot the standard curve using SigmaPlot software (or other software which can perform four-parameter logistic regression models), with standard concentration on the x-axis and percentage of absorbance (see calculation below) on the y-axis. Draw the best-fit curve through the standard points.

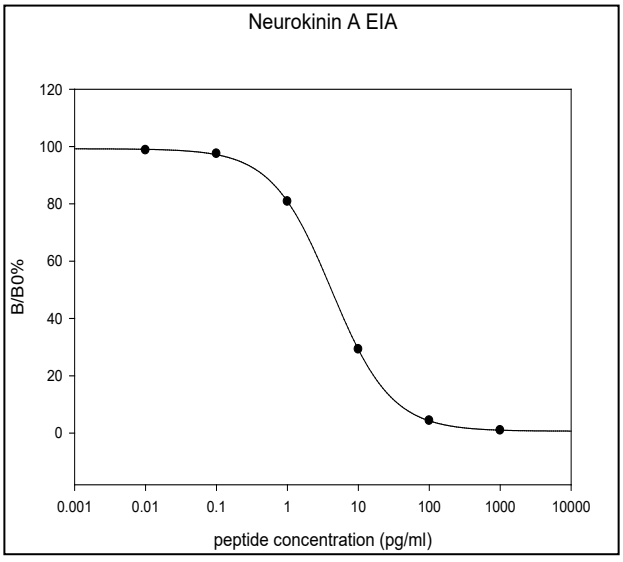
Percentage absorbance =

$$(B - \text{blank OD})/(B_o - \text{blank OD})$$

B = OD of sample or standard
B_o = OD of zero standard (total binding)

Typical Data

Standard curve(s) is for demonstration only. Standard curve(s) must be run with each assay.



Product Profile

Sensitivity: The minimum detectable concentration of Neurokinin A is 0.8 pg/mL.

Reproducibility:
Intra-Assay: CV <10%
Inter-Assay: CV <15%

Detection range:
0.1–1,000 pg/mL

Specificity

Cross Reactivity: This kit shows no cross-reactivity with any of the cytokines tested: Ghrelin, Nesfatin, Angiotensin II, NPY, and APC.

References

1. Dornan, W.A. et al., Site-specific effects of intracerebral injections of three neurokinins (neurokinin A, neurokinin K, and neurokinin gamma) on the expression of male rat sexual behavior. Physiol. Behav., **54**(2), 249-258 (1993).

2. Carter, M.S., and Krause, J.E., Structure, expression, and some regulatory mechanisms of the rat preprotachykinin gene encoding substance P, neurokinin A, neuropeptide K, and neuropeptide gamma. J. Neurosci., **10**(7), 2203-2214 (1990).

Appendix

Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure a brief spin of Item C and dissolve the powder thoroughly with gentle mixing.
Low signal	Too brief incubation times	Ensure sufficient incubation time; Procedure, step 2 may change to overnight
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the standard at <–20 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light
	Stop solution	Stop solution should be added to each well before measurement.

SA,ANK,TD,KJ,MAM, CY 03/21-1