Mouse Adenosine Receptor A2a ELISA Kit

Catalog Number: A312062

Mouse Adenosine Receptor A2a ELISA Kit is a 90 minute sandwich Enzyme-Linked Immunosorbent Assay (sELISA) designed for the in vitro quantitative determination of mouse Adenosine Receptor A2a in serum, plasma, and other biological fluids.

Size: 96T

Sensitivity: 9.41 ng/L

Range: 150-2400 ng/L

Storage: Store entire kit at +4°C. Do not use past expiration date!

This protocol booklet must be read in its entirety before use.

This product is for research use only. It is not intended for diagnostic or therapeutic use.

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PRINCIPLE OF ASSAY

Mouse Adenosine Receptor A2a ELISA Kit (A312062) employs the sandwich enzyme immunoassay technique for the quantitative measurement of mouse Adenosine Receptor A2a in serum, plasma or other biological fluids. An antibody specific for Adenosine Receptor A2a has been pre-coated onto a 96-well microtiter plate. The standards and test samples are added into the wells and the Adenosine Receptor A2a present in each sample is bound to the wells by the immobilized antibody. Biotinylated Anti-Adenosine Receptor A2a Antibody, which also binds the Adenosine Receptor A2a present in each sample, and Streptavidin-HRP, which binds the Biotinylated Anti-Adenosine Receptor A2a Antibody, are added and the microtiter plate is incubated. Following incubation, unbound Biotinylated Anti-Adenosine Receptor A2a Antibody and unbound Streptavidin-HRP are removed by washing, and two substrate solutions are added to the wells. Color develops in proportion to the amount of Adenosine Receptor A2a captured in each well. The color development is stopped by addition of stop solution which changes the color from blue to yellow and the intensity of the color is then measured. The concentration of Adenosine Receptor A2a in the samples can then be calculated by reading the O.D. absorbance at 450nm in a microplate reader and referring to the standard curve.

LIMITATIONS

- Do not use this kit past the expiration date.
- Do not mix or substitute reagents from different lots or different sources.
- This instructions manual must be strictly followed.
- Variations in sample collection, sample processing, and sample storage may cause sample value differences.
- Any variation in operating procedure, including the operator, pipetting technique, washing technique, incubation time, incubation temperature, and kit age, can cause variation in binding and result in sample value differences.

KIT COMPONENTS AND STORAGE

Store the unopened kit at +4°C. Do not use past expiration date!

Item	Quantity	Storage
Pre-Coated 96 Well Microplate	12 x 8 Well Strips	+4°C
Standard Solution	500µl	+4°C
Standard Diluent	3ml	+4°C
Biotinylated Detection Antibody	1ml	+4°C
Streptavidin-HRP	6ml	+4°C
Wash Buffer (25X)	20ml	+4°C
Substrate Solution A	6ml	+4°C
Substrate Solution B	6ml	+4°C
Stop Solution	6ml	+4°C
Plate Sealers	5 Adhesive Strips	-
Foil Pouch	1 Zip-Sealed Pouch	-

REQUIRED MATERIALS

The following materials are not included in the kit, but are required to run this assay:

- Microplate reader capable of measuring absorbance at 450nm ± 10nm.
- Squirt bottle, multichannel pipette reservoir, or automated microplate washer.
- Graph paper or computer software capable of generating logarithmic functions.
- Multi- and single-channel pipettes and sterile pipette tips.
- Test tubes to prepare standards and samples.
- Deionized or distilled water.
- Absorbent paper towels.
- 37°C incubator.

PRECAUTIONS

- The Stop Solution provided with this kit is an acid solution. Avoid any skin and eye contact. In case of contact, wash thoroughly with water.
- Wear protective equipment such as gloves, masks, googles, and lab coats.
- Refer to the SDS on our website prior to use.
- Substrate Solution B is light sensitive. Avoid exposure for prolonged periods of time.
- To avoid cross-contamination, change pipette tips between additions of each standard level, each sample additions, and between reagent additions.
- To avoid cross contamination, use separate reservoirs for each reagent.
- Ensure reagent addition to plate wells is uninterrupted.
- Ensure consistent pipetting order and rate of addition from well-to-well i.e., all reagents should be added to the plate in the same order as the standards and samples were and at the same speed.
- To ensure accurate results, proper adhesion of the plate sealer during the incubation step is necessary.

SAMPLE COLLECTION AND STORAGE

<u>The sample collection and storage conditions listed below are intended as general guidelines.</u>

Serum – Samples should be collected in serum separator tubes (SST). Allow blood samples to clot for 30 minutes at room temperature. After clot formation, centrifuge samples at 1000 x g for 15 minutes and collect serum. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze/thaw cycles.

Plasma – Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples at 1000 x g for 15 minutes within 30 minutes of collection. Plasma samples can be can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze/thaw cycles.

Note: Citrate plasma was not validated for use in this assay.

Urine / Ascites / Cerebrospinal Fluid – Collect samples in sterile tubes. After collection, centrifuge samples at 1000 x g for 15 minutes. Collect the supernatant without sediment. Samples can be can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze/thaw cycles.

Tissue – Rinse tissues thoroughly in ice-cold Phosphate Buffered Saline (pH 7.4) to remove excess blood and weigh before homogenization. Mince tissues and homogenize them in PBS (1g tissue / 9ml PBS) with a glass homogenizer on ice. To further break the cells, the sample may be treated with an ultrasonic cell disrupter or subjected to freeze/thaw cycles. After homogenization, centrifuge the samples at 5000 x g for 5 minutes and obtain the supernatant. Samples can be assayed immediately or aliquoted and stored at -80°C. Avoid repeated freeze/thaw cycles.

IMPORTANT NOTES

Sample concentrations should be estimated before being used in the assay. If the sample concentration is not within the range of the standard curve, users must contact us to determine the optimal sample for their experiments.

Samples to be used within 48 hours should be stored at +4°C. For longer term storage, samples should be aliquoted and stored at -20°C (for up to 1 month) or -80°C (for up to 6 months). Avoid repeated freeze/thaw cycles.

Samples should be brought to room temperature before starting the assay.

Collect the supernatants carefully. If sedimentation occurs during storage, remove by centrifugation. Centrifuge tubes to collect the sample before use.

Samples containing Sodium Azide (NaN_3) cannot be used in this assay. NaN_3 inhibits the activity of Horseradish Peroxidase (HRP) which is used in the detection step.

Hemolysis can greatly impact the validity of test results. Take care to minimize hemolysis.

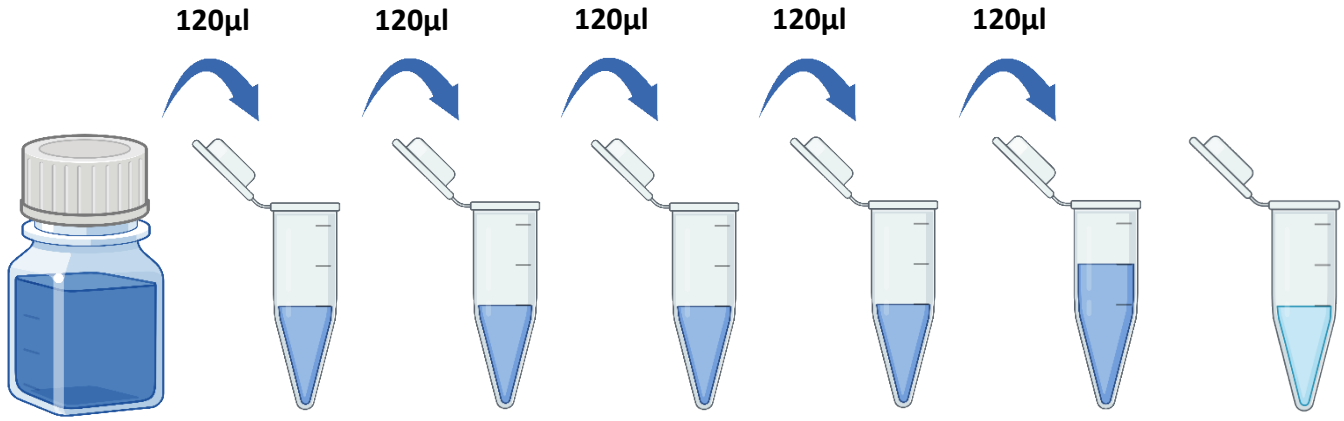
Samples cannot be diluted for use with this kit. Owing to the material used to prepare the kit, sample matrix interference may falsely depress the specificity and accuracy of the assay.

REAGENTS PREPARATION

Bring all reagents to room temperature before use.

Standard – Add 120µl of the Standard Solution (4800ng/L) to 120µl of Standard Diluent to produce a stock solution of 2400ng/L. Allow the standard to sit for 15 minutes with gentle agitation prior to making dilutions. Use the stock solution to prepare duplicate 1:2 dilution series (shown below) of 1200ng/L, 600ng/L, 300ng/L, and 150ng/L. Each dilution, pipette 120µl of the previous standard into a tube along with 120µl of Standard Diluent. Mix each tube thoroughly before the next transfer. The 2400ng/L standard serves as the high standard. The Standard Diluent serves as the zero standard (0ng/L).

Note: Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well.



Standard	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Blank
4800ng/L	2400ng/L	1200ng/L	600ng/L	300ng/L	150ng/L	0ng/L

Wash Buffer – If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20ml of Wash Buffer (25X) concentrate to 480ml of deionized or distilled water to prepare 500ml of Wash Buffer.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. We recommend that all standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents, samples, and working standards as directed in the previous sections.

2. Determine the number of microplate strips required for the assay and insert them into the plate frame. Unused strips should be stored in the foil pouch at +4°C.

3. Add 50µl of standard to the standard wells.

4. Add 40µl of samples to the sample wells.

   Note: A plate layout is provided to record standards and samples assayed.

5. Add 10µl of Biotinylated Detection Antibody to sample wells.
   Note: Do not add biotinylated detection antibody to the standard wells because the standard solution already contains biotinylated detection antibody.

6. Add 50µl of Streptavidin-HRP to sample wells and standard wells (**but not to the blank control well**). Mix well. Cover with the adhesive plate sealer provided. Incubate for 60 minutes at 37°C.

7. Remove the plate sealer and wash the plate 5 times with Wash Buffer. Soak wells with 300µl Wash Buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with Wash Buffer. 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. Blot the plate onto paper towels or other absorbent material.

8. Add 50µl of Substrate Solution A to each well and then add 50µl of Substrate Solution B to each well. Cover with a new adhesive plate sealer and incubate for 10 minutes at 37°C **in the dark.**

9. Add 50µl of Stop Solution to each well. The color in the wells should change from blue to yellow immediately. Note: 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 (O.D. value) of each well within 10 minutes, using a microplate reader set to 450 nm.

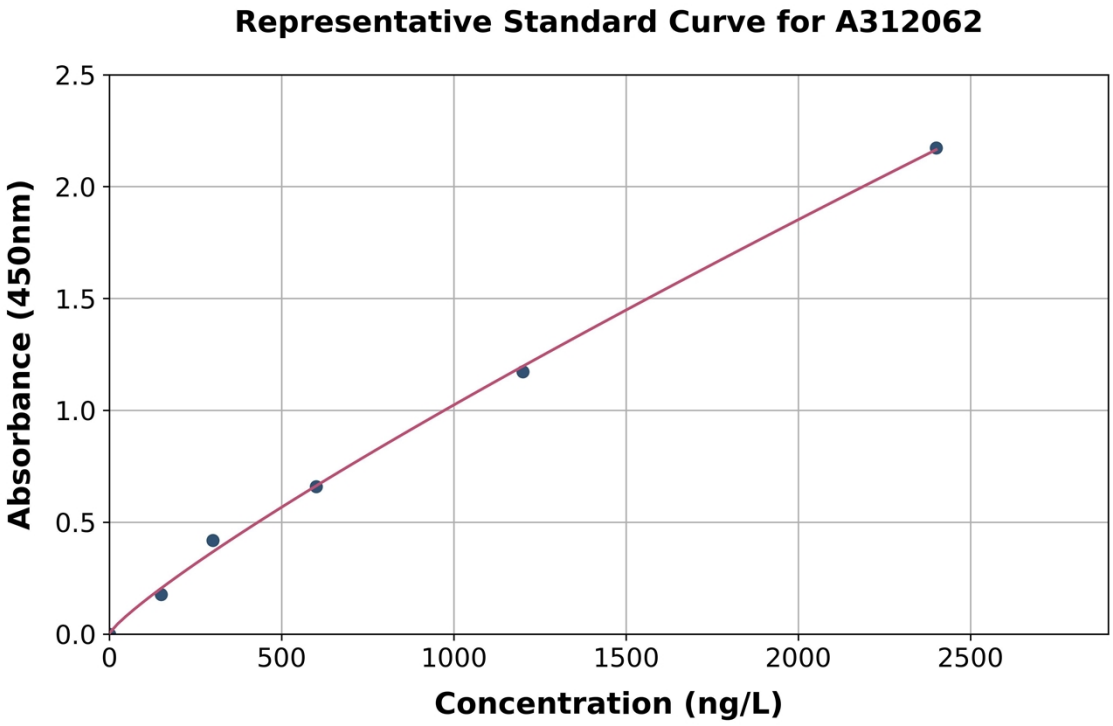
CALCULATION OF RESULTS

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

Create a standard curve using computer software capable of generating a four-parameter logistic (4-PL) curve fit. Alternatively, you can create a standard curve by plotting the average absorbance for each standard on the y-axis (vertical) against the known standard concentrations on the x-axis (horizontal) and draw a best fit curve through the points on the graph.

TYPICAL DATA

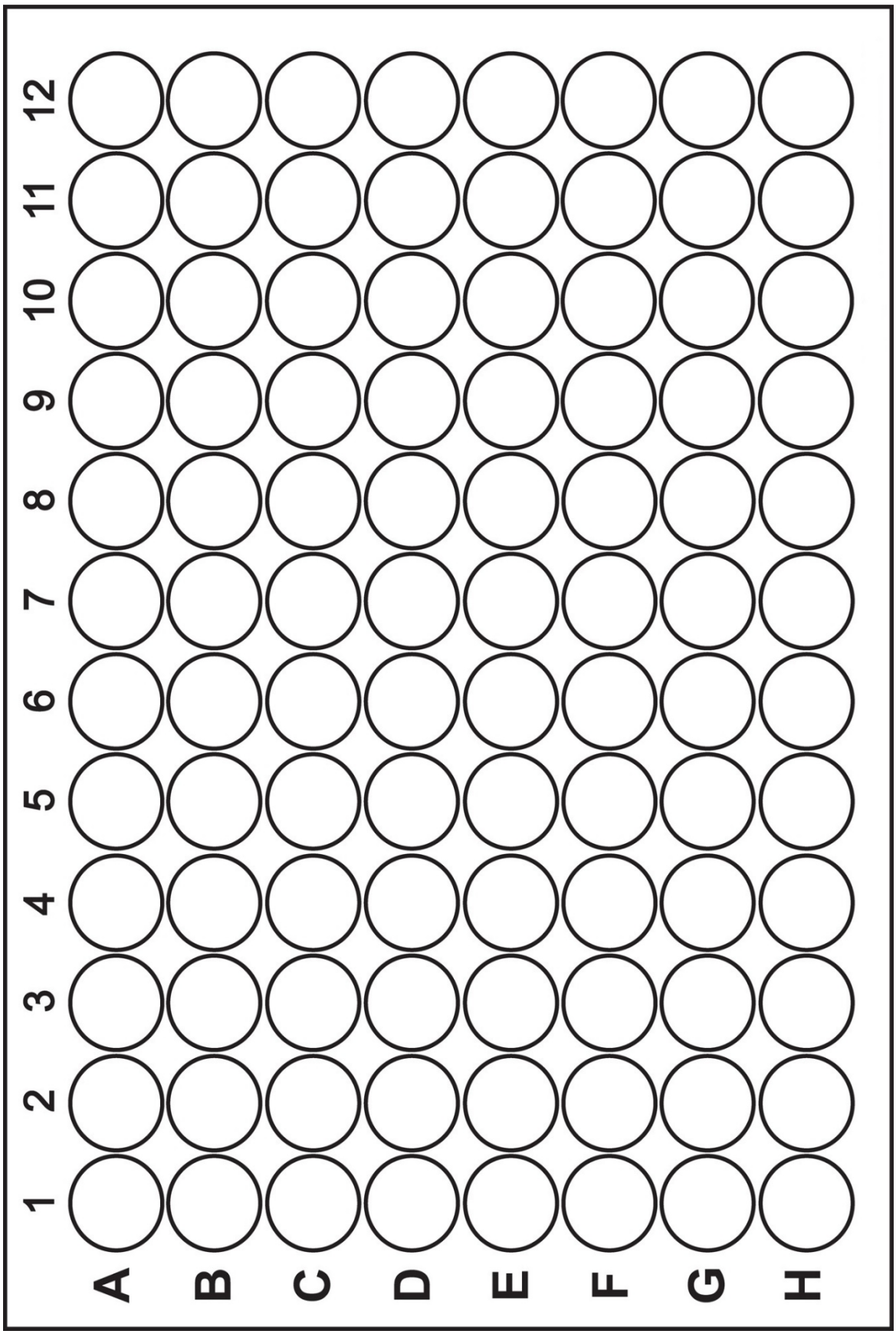
This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/L)	O.D.	Average	Corrected
0ng/L	0.05 0.062	0.056	-
150ng/L	0.223 0.243	0.233	0.177
300ng/L	0.416 0.535	0.476	0.419
600ng/L	0.675 0.755	0.715	0.659
1200ng/L	1.277 1.182	1.23	1.173
2400ng/L	2.282 2.179	2.231	2.174

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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