Rat Platelet Activating Factor ELISA Kit

Catalog Number: A80109

Rat Platelet Activating Factor ELISA Kit is a competitive Enzyme-Linked Immunosorbent Assay (cELISA) designed for the in vitro quantitative determination of rat Platelet Activating Factor in serum, plasma, tissue homogenates, and other biological fluids.

Size: 96T

Sensitivity: 0.094 ng/ml

Range: 0.156-10 ng/ml

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.

TABLE OF CONTENTS

PRINCIPLE OF ASSAY

Rat Platelet Activating Factor ELISA Kit (A80109) employs the competitive enzyme immunoassay technique for the quantitative measurement of rat Platelet Activating Factor in serum, plasma, tissue homogenates, and other biological fluids. The 96-well microtiter plate has been pre-coated with Platelet Activating Factor antigen. During the incubation, Platelet Activating Factor present in the samples or standards competes with the fixed amount of immobilized Platelet Activating Factor for binding sites on the Biotinylated Anti-Platelet Activating Factor Antibody. The more Platelet Activating Factor present in a sample or standard, the less Biotinylated Anti-Platelet Activating Factor Antibody that binds to the plate. Following incubation, unbound Biotinylated Anti-Platelet Activating Factor Antibody is removed by washing, and an HRP-Streptavidin conjugate is added to the wells and the microtiter plate is incubated. Following incubation and washing, TMB substrate solution is then used to visualize the HRP enzymatic reaction by catalysis to produce a blue-coloured product that changes to yellow after addition of acidic stop solution. The density of yellow is inversely proportional to the amount of Platelet Activating Factor present in each sample or standard. The concentration of Platelet Activating Factor 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 / -20°C
Lyophilized Standard	2 Vials	+4°C / -20°C
Sample Dilution Buffer	20ml	+4°C
Biotinylated Detection Antibody (100X)	60µl	+4°C (Avoid light!)
Antibody Dilution Buffer	10ml	+4°C
HRP-Streptavidin Conjugate (SABC) (100X)	120µl	+4°C (Avoid light!)
SABC Dilution Buffer	10ml	+4°C
TMB Substrate	10ml	+4°C (Avoid light!)
Stop Solution	10ml	+4°C
Wash Buffer (25X)	30ml	+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.
- Test tubes and Eppendorf tubes to prepare standards and samples.
- Multi- and single-channel pipettes and sterile pipette tips.
- Deionized or distilled water.
- Absorbent paper towels.

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.
- TMB Substrate 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 steps is necessary.

SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions listed below are intended as general guidelines.

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: Avoid hemolysis and high cholesterol samples.

Cell Culture Supernatant – Remove particulates by centrifugation at 500 x g for 5 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.

Note: Avoid using any reagents containing NP-40 lysis buffer, Triton X-100 surfactant, or DTT as these components can significantly inhibit the performance of this kit. We recommend using 50mM Tris, pH 7.3, with 0.9% NaCl and 0.1% SDS.

Cell Lysates – Solubilize cell in lysis buffer and allow to sit on ice for 30 minutes. Centrifuge tubes at 14,000 x g for 5 minutes to remove insoluble material. Aliquot the supernatant into a new tube and discard the remaining whole cell extract. Quantify total protein concentration using a total protein assay. Assay immediately or aliquot and store at -20°C. Avoid repeated freeze/thaw cycles.

Note: Avoid using any reagents containing NP-40 lysis buffer, Triton X-100 surfactant, or DTT as these components can significantly inhibit the performance of this kit. We recommend using 50mM Tris, pH 7.3, with 0.9% NaCl and 0.1% SDS.

Other Biological Fluids – Centrifuge samples at 1000 x g for 20 minutes at 1000×g at 4°C. Collect the supernatant and carry out the assay immediately.

IMPORTANT NOTES

Sample concentrations should be estimated before being used in the assay and a proper dilution factor should be selected to make the diluted target protein concentration fall in the optimal detection range of this kit.

Samples to be used within 5 days 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.

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

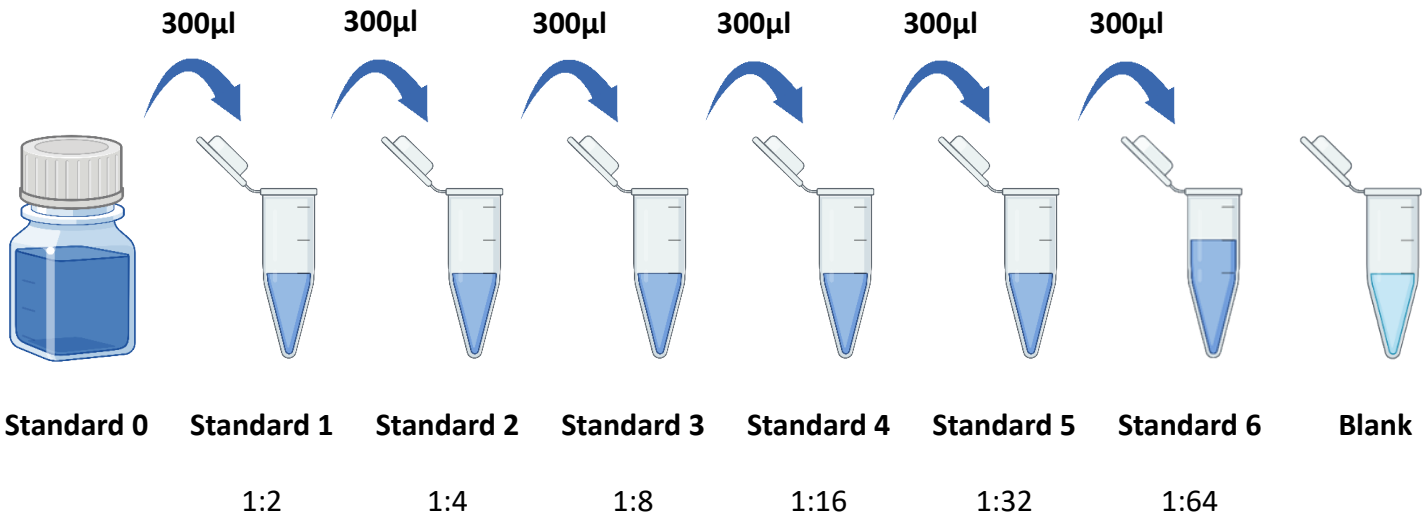
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer – If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30ml of Wash Buffer (25X) concentrate to 720ml of deionized or distilled water to prepare 750ml of Wash Buffer.

Standards – Add 1ml of Sample Dilution Buffer to one Lyophilized Standard vial and label as Standard 0. Keep the tube at room temperature for 10 minutes and mix thoroughly. Note: If the concentration of the Lyophilized Standard vial is higher than the detection range of the kit, it needs to be diluted appropriately. Label 7 microfuge tubes as Standard 1, Standard 2, Standard 3, Standard 4, Standard 5, Standard 6, and Blank, respectively. Use Standard 0 as the stock solution to prepare a 1:2 dilution series (shown below) of 1:2, 1:4, 1:8, 1:16, 1:32, and 1:64. Each dilution, pipette 300µl of the previous standard into the next tube along with 300µl of Sample Dilution Buffer. Mix each tube thoroughly before the next transfer. Standard 0 serves as the high standard. The Sample Dilution Buffer serves as the zero standard

Note: Standards should be used within 2 hours of preparation.



HRP-Streptavidin Conjugate (SABC) – Calculate the total volume of HRP-Streptavidin Conjugate (SABC) working solution required to run the assay. The assay requires 100µl per well and it is recommended to prepare ~ 200µl more than the total volume required. i.e. (100µl × number of wells) + 200µl. Dilute the HRP-Streptavidin Conjugate (SABC) with SABC Dilution Buffer at 1:100 and mix thoroughly.

Note: HRP-Streptavidin Conjugate (SABC) should be used within 30 minutes of preparation.

Biotinylated Detection Antibody – Calculate the total volume of Biotinylated Detection Antibody working solution required to run the assay. The assay requires 50µl per well and it is recommended to prepare ~ 200µl more than the total volume required. i.e. (50µl × number of wells) + 200µl. Dilute the Biotinylated Detection Antibody with Antibody Dilution Buffer at 1:100 and mix thoroughly.

Note: Biotinylated Detection Antibody should be used within 30 minutes of preparation.

If the Biotinylated Detection Antibody is supplied lyophilized instead of 100X concentrate, you will need to reconstitute it before preparing the Biotinylated Detection Antibody working solution. Centrifuge vial at 2000 x g for 1 minute. Reconstitute with 70µl deionized or distilled water. Store reconstituted Biotinylated Detection Antibody at +4°C.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. We recommend that all standards and samples be assayed in duplicate. When diluting samples and reagents, it is critical that they're mixed completely and evenly. Before adding TMB into the wells, equilibrate TMB Substrate for 30 minutes at 37°C.

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. Wash the plate two times with Wash Buffer. Soak wells with 350µl of Wash Buffer for 1-2 minutes each time.

   Note: 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. Do not let the wells dry completely at any time!

4. Add 50µl of standard solutions to the standard wells.

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

5. Add 50µl of samples to the sample wells.

   Note: All samples must be properly diluted in order to produce sample values within the dynamic range of the assay.

6. Immediately add 50µl of Biotinylated Detection Antibody working solution to each well. Gently tap the plate to ensure thorough mixing for 1 minute.

7. Cover with an adhesive plate sealer provided and incubate at 37°C for 45 minutes.

8. Remove the plate sealer, aspirate the liquid from the plate, and wash the plate three times with Wash Buffer. Soak wells with 350µl of Wash Buffer for 1-2 minutes each time.

9. Add 100µl of HRP-Streptavidin Conjugate (SABC) working solution to each well.

10. Cover with a new adhesive plate sealer and incubate at 37°C for 30 minutes.

11. Remove the plate sealer, aspirate the liquid from the plate, and wash the plate five times with Wash Buffer. Soak wells with 350µl of Wash Buffer for 1-2 minutes each time.

12. Add 90µl of TMB Substrate to each well. Cover with a new adhesive plate sealer and incubate at 37°C in the dark for 10-20 minutes.

    Note: This incubation time is for reference only, the optimal reaction time should be determined by the end-user and can be shortened or extended according to the actual color change. The reaction may be terminated when a gradient is apparent in the standard wells. Do not exceed 30 minutes!

13. 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.

14. Determine the optical density (O.D. value) of each well immediately using a microplate reader set to 450 nm.

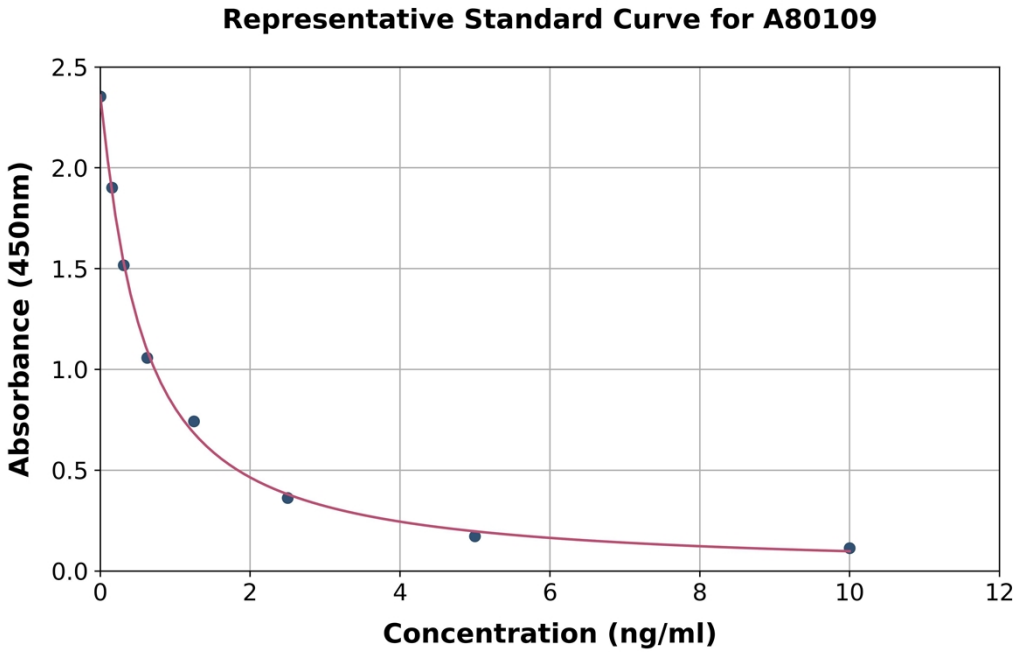
CALCULATION OF RESULTS

Average the duplicate readings for each standard 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/ml)	O.D.	Average
0.00	2.32 2.39	2.35
0.16	1.88 1.93	1.90
0.31	1.50 1.54	1.52
0.63	1.04 1.07	1.06
1.25	0.73 0.75	0.74
2.50	0.36 0.37	0.36
5.00	0.17 0.17	0.17
10.00	0.11 0.12	0.11

PRECISION

Intra-Assay Precision (Precision within an assay)

Samples with known low, medium, and high concentrations of rat Platelet Activating Factor were tested twenty times on the same plate to assess intra-assay precision.

Intra-Assay Precision: CV <8%

Inter-Assay Precision (Precision between assays)

Samples with known low, medium, and high concentrations of rat Platelet Activating Factor were tested twenty times on three different plates to assess inter-assay precision.

Inter-Assay Precision: CV <10%

RECOVERY

The recovery of rat Platelet Activating Factor spiked to different levels in samples throughout the range of the assay in various matrices was evaluated. The recovery rates were calculated by comparing the measured value to the expected amount of Rat Platelet Activating Factor in the samples.

Sample Type	n	Recovery Range (%)	Average (%)
Serum	5	85% - 102%	92%
EDTA Plasma	5	86% - 103%	96%
Heparin Plasma	5	87% - 103%	95%

LINEARITY

The assess the linearity of the assay, samples spiked with rat Platelet Activating Factor in various matrices were diluted with Sample Dilution Buffer to produce samples with values within the dynamic range of the assay. The recovery rates were calculated by comparing the measured value to the expected amount of Rat Platelet Activating Factor in the samples.

Sample Type	n	1:2	1:4	1:8
Serum	5	95-105%	90-101%	90-104%
EDTA Plasma	5	82-99%	92-100%	89-97%
Heparin Plasma	5	81-94%	82-96%	83-96%

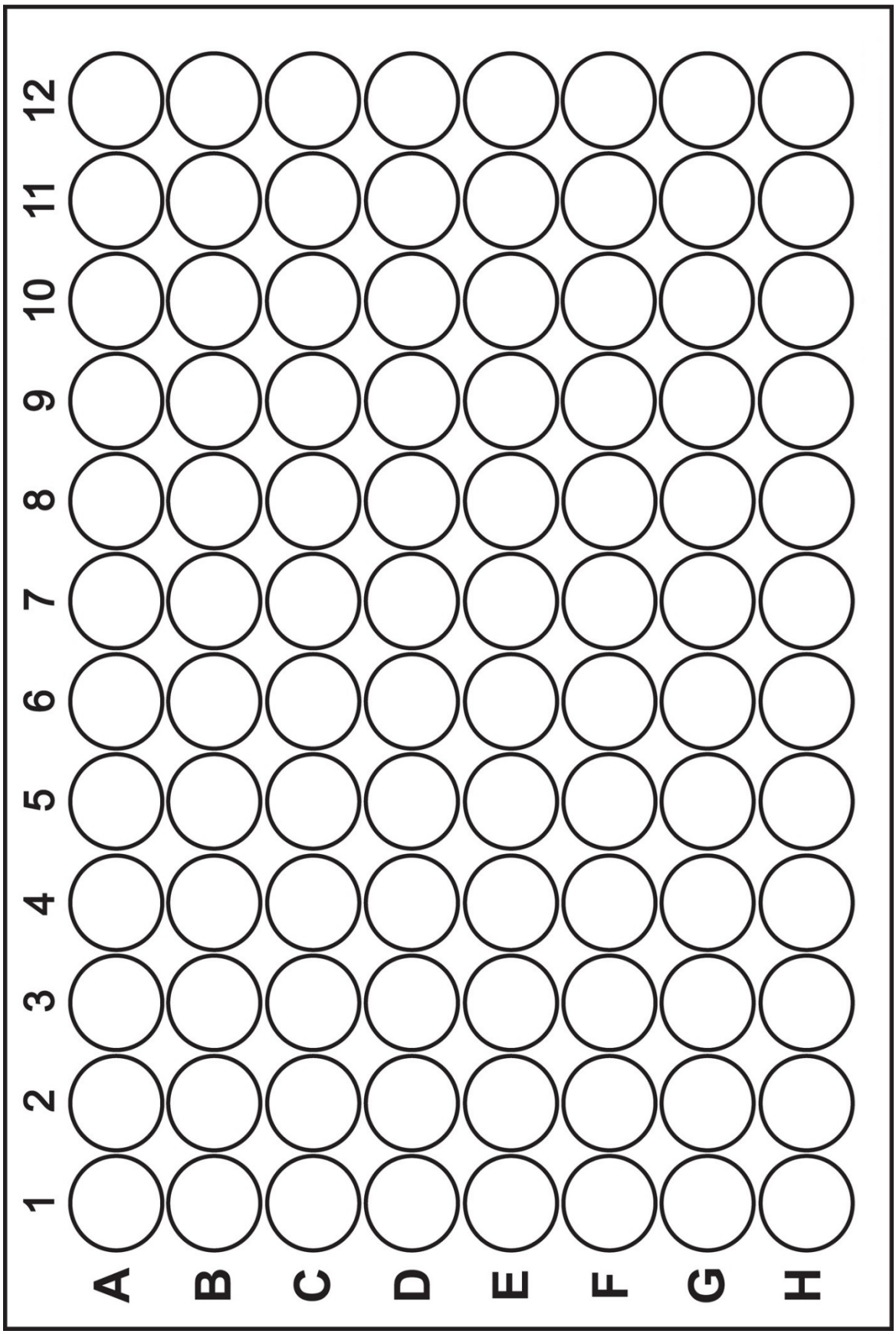
STABILITY

The stability of an ELISA kit is determined by the rate of loss of activity. The loss rate of this ELISA kit is less than 5% within the expiration date under appropriate storage conditions.

ELISA kits (n=5)	37°C for 1 month	4°C for 6 months
Average	80%	95-100%

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



CONTACT US

For further information or technical support, please visit us online at www.antibodies.com or contact us directly at:

Antibodies.com LLC

Helix Center
1100 Corporate Square Drive
St. Louis
MO 63132
USA

Tel.: +1 (314) 370-6046

Antibodies.com Europe AB

Kammakargatan 47
111 24
Stockholm
Sweden

Tel.: +46 (0)8-525 070 54

Antibodies.com Limited

8 Station Court
Station Road
Cambridge
CB22 5NE
United Kingdom

Tel.: +44 (0)1223 298 875

Email Addresses

Customer Service:	info@antibodies.com
Quotation:	quotes@antibodies.com
Orders:	orders@antibodies.com
Technical Support:	technical@antibodies.com