ELISA PRODUCT INFORMATION & MANUAL

Monkeypox Virus A29 ELISA Kit (Colorimetric)

NBP3-20174

Sample insert for reference use only

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

PRINCIPLE OF THE ASSAY

The principle of this ELISA kit is based on the Sandwich Enzyme-Linked Immunosorbent Assay (ELISA). The 96-well strip plate is precoated with a monoclonal antibody specific for Monkeypox Virus A29. Standards and Monkeypox Virus A29 present in the sample is bound by the immobilized antibody. After washing, a horseradish peroxidase conjugated anti-Monkeypox Virus A29 antibody is added, producing an antibody-antigen-antibody "sandwich complex". Following a wash to remove any unbound antibody -enzyme reagent, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Monkeypox Virus A29 bound in the initial step. The color development is stopped and the intensity of the color is measured at 450 nm.

KIT CONTENTS AND STORAGE

The kit, if unopened, is stable at 2-8℃ for 6 months upon receipt.

<table>
<tr><th>Components</th><th>Amt</th><th>Preparation instructions</th><th>storage</th></tr>
<tr><td>Pre-coated microplate</td><td>1plate (96 tests)</td><td>Take the microplate strips as needed, and put the unused strips back to the vacuum bag. It is best to vacuumize them.</td><td>Vacuum storage can store at 2-8℃ until expiration date and opened package store at 2-8℃ for one month.</td></tr>
<tr><td>Detection Antibody</td><td>1 vial</td><td>Dilute at 1:2000 with 1×dilution buffer for 10 minutes before use. Dilute fresh as needed.</td><td rowspan="7">Primary liquid are stable at 2 - 8℃ until expiration date.
To be reconstituted, the working fluid is used within the working day and discard. So dilute fresh as needed.</td></tr>
<tr><td>Standard</td><td>1 bottle</td><td>Add 1 mL of 1×Dilution Buffer to the lyophilized standard bottle, briefly vortex to mix completely and prepare a standard stock solution.</td></tr>
<tr><td>20 × Dilution Buffer</td><td>1 bottle</td><td rowspan="2">If crystals have formed in the 20 ×concentrated solution, bring to room temperature and mix until dissolved. Dilute the 20× concentrated solution to 1× working solution with deionized water. For example, make 400 ml of 1× Wash Buffer by adding 20 mL of 20× Wash Buffer to 380 mL of deionized water. Dilute fresh as needed.</td></tr>
<tr><td>20 × Wash Buffer</td><td>1 bottle</td></tr>
<tr><td>Color Reagent A</td><td>1 bottle</td><td rowspan="2">Color Reagents A and B should be mixed together in equal volumes within 10 minutes before use. Take care not to contaminate the Color Reagent. If the mixed color reagent is blue, DO NOT USE</td></tr>
<tr><td>Color Reagent B</td><td>1 bottle</td></tr>
<tr><td>Stop Solution</td><td>1 bottle</td><td>Dilute sulfuric acid. Use directly according to the use volume. Pay attention to safety when using</td></tr>
</table>

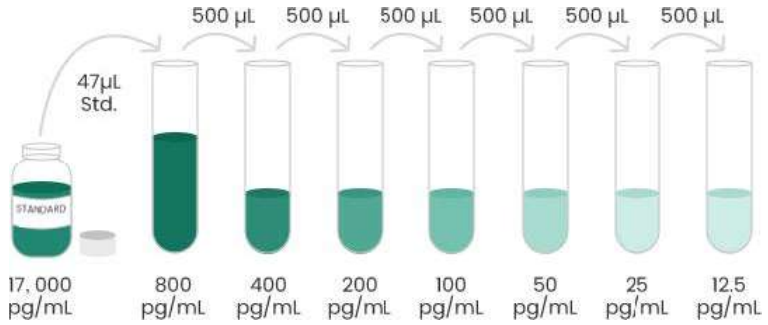
ASSAY PROCEDURE

1. Plate Set-up

- Bring all reagents to room temperature (22-28℃) equilibration (at least 30 minutes) before use. If crystals have formed in buffer solution, warm to room temperature and mix gently until the crystals have completely dissolved.
- Determine the number of wells for the assay run. Remove unused microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
- Add 300 µL1×Wash Buffer to each well and let stand for about 2 minutes. Aspirate or dump the liquid and pat dry on a paper towel, wash twice in this way.

2. Incubation with standard and samples [Volume: 100 µL Time: 2 hours]

- Make standard curve: Prepare 1000 µL of the 800 pg/mL top standard by adding 47 µL of the standard stock solution in 953 µL of 1× Dilution Buffer. Perform six two-fold serial dilutions of the 800 pg/mL top standard in 6 separate tubes using 500 µL 1× Dilution Buffer as the diluents: after mixing the 800 pg/mL top standard, pipette 500 µL into the next tube, and so on. 1× Dilution Buffer serves as the zero standard (0 pg/mL).Ensures each assay has a standard curve. DO NOT USE the standard curve on other plates or other days.



- Add 100 µL standard and your test samples per well. Cover/seal the plate and incubate for 2 hours at room temperature.
- Add 300 µL 1 × Wash Buffer to each well and let stand for about 2 minutes. Aspirate or dump the liquid and pat dry on a paper towel, wash wells 3 times in this way. Improper washes may lead to falsely elevated signals and poor reproducibility.

3. Incubation with Secondary Antibody [Volume: 100 µL Time: 1 hour]

- Add 100 µL of detection antibody working solution into each well, mix gently.
- Cover/seal the plate and incubate for 1 hour at room temperature.
- Removal the liquid in the wells and repeat the aspiration/wash as in Step 2.

4. Incubation with Substrate [Volume: 100 µL Time: about 20 minutes]

- Add 100 µL of Substrate Solution (the mixture of Color Reagents A and B) to each well, mix gently.
- Incubate for 20 minutes at room temperature. Protect from light. (According to the color of sample and the control antibody, the chromogenic time should been shortened or prolonged.)

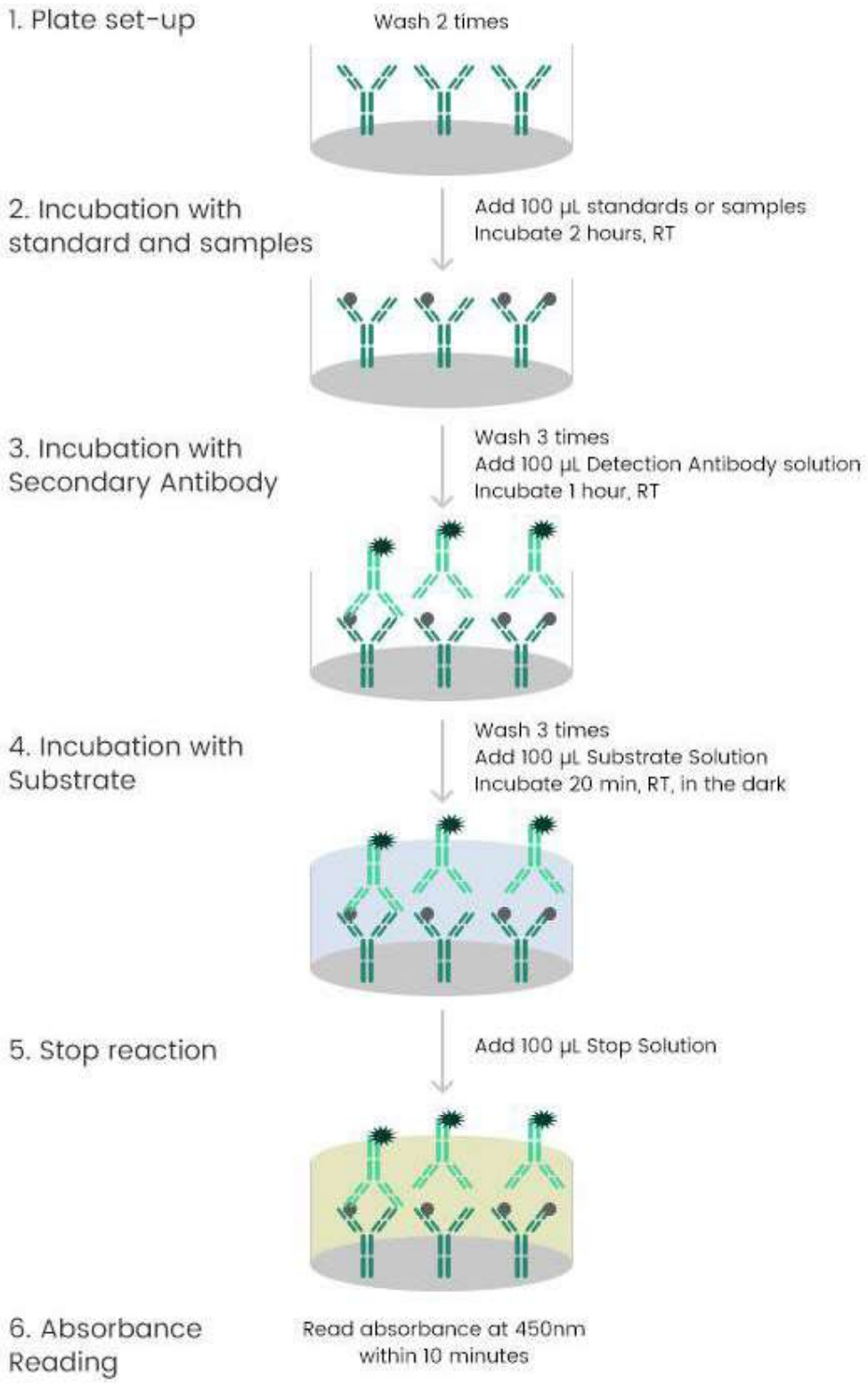
5. Stop reaction

- Add 100 µL of Stop Solution to each well.
- Tap gently the plate to ensure it is well mixed.

6. Absorbance Reading

- Read absorbance of the entire plate at 450nm wavelength within 10 minutes after adding the stop solution.

ASSAY PROCEDURE SUMMARY



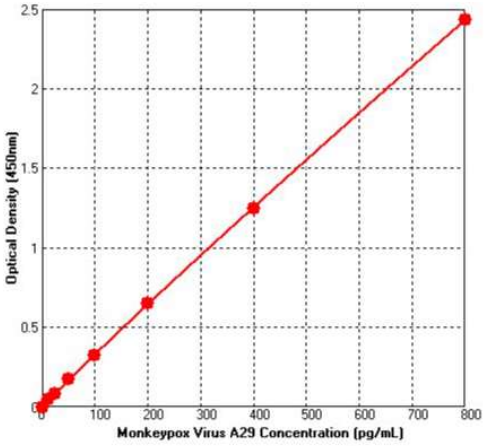
CALCULATION OF RESULTS

1. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
2. Calculate the mean absorbance for each standard and sample, subtract average zero standard optical density.
3. The data been calculated by 4-parameter logistics curve-fitting algorithm.

TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be run with each assay.

Concentration (pg/mL)	Zero standard subtracted OD
0	0
12.5	0.046
25	0.081
50	0.173
100	0.321
200	0.651
400	1.244
800	2.433



PERFORMANCE CHARACTERISTICS

Precision:

Intra-assay Precision (Precision within an assay) - Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) - Three samples of known concentration were tested in three separate assays to assess inter-assay precision.

	Intra -assay Precision			Inter -assay Precision		
Sample	1	2	3	1	2	3
N	20	20	20	3	3	3
Mean (pg/mL)	130	190	392	106	201	395
SD	4.19	5.16	13.6	4.41	14.79	26.51
CV (%)	3.2%	2.7%	3.5%	4.2%	7.4%	6.7%

Recovery: The recovery of Monkeypox Virus A29 spiked to different levels throughout the range of the assay in related matrices was evaluated.

Sample	Average % Recovery	Range
Cell culture supernates (n=3)	112	111 -114%
Serum (n=3)	117	112 -126%

Linearity:

		Serum	Cell culture supernates
1:2	recovery of detected	98%	106%
1:4	recovery of detected	102%	94%
1:8	recovery of detected	113%	87%
1:16	recovery of detected	119%	80%

Sensitivity: 1.09 pg/mL. Which was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

PRECAUTIONS

1. This kit is **for research use only** and is not for use in diagnostic or therapeutic procedures.
2. The kit should not be used beyond the expiration date.
3. Do not mix reagents from different lots.
4. The kit is designed and tested to detect the application which shown in the manual. The use of this kit for other purpose should be verified carefully by the end user.

SAFETY INSTRUCTIONS

1. The Stop Solution provided with this kit is an acid solution. Take care when using the reagent to avoid the risks.
2. All biological materials should be handled and discarded as potentially hazardous following local laws and regulations.
3. Personal protective equipments such as lab coats, gloves, surgical masks and goggles are necessary in experiments for safety reasons.

TECHINICAL TIPS

1. Bring all reagents and samples to room temperature before use.
2. Samples should be thawed completely and mixed well prior to analysis. Avoid repeated freeze-thaw cycles of frozen samples.
3. Use a new disposable reagent reservoir and new disposable pipette tips for each transfer to avoid cross-contamination.
4. Read the absorbance of each well within 10 minutes after adding the stop solution.