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# Polio RDRP Antibody ELISA Kit

**Cat. No. E20142012 (One 96-well Plate, 12 x 8 strips)**

GeneScan Diagnostics' AmeriDx® ELISA Kit is designed for the quantification of poliovirus RDRP antibody and other polio virus antibodies in human serum or other human body fluids. The assay is sensitive, accurate and reliable.

***It is highly recommended that this instruction sheet be read in its entirety before using this product. Do not use this set beyond the expiration date.***

## Materials Provided

1. Polio virus antigen coated ELISA plate
2. RDRP antibody Standards (in house Antibody extraction):
   - 40 pg/mL
   - 80 pg/mL
   - 160 pg/mL
3. Negative Control (2)
4. Goat anti human IgG/A/M HRP (lyophilized powder)
5. Substrate Solution (1X)
6. Wash Buffer (10X)
7. Assay Diluent (10X)
8. Stop Solution (1)

## Introduction

Poliovirus can cause life-threatening diseases once infected in the human body without vaccination. Most of the people in the world are immunized with polio virus vaccine by either OPV or IPV during their early age. However the antibody titer maintain around 20 years after the first immunization, after that the infection risk comes back. A boost shot of polio vaccine is needed to re-activate the protection mechanism. The kit is designed to monitor the antibody titer in human serum or other body fluids.

## Principle of the Test

GeneScan Diagnostics' AmeriDx® ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay. A polio virus antigen first coated on a 96-well plate. Standards, positive controls, negative controls and samples are added to the wells, and antibody in the samples binds to the immobilized capture antigen. Next, a HRP conjugated goat anti human IgG/A/M antibody is added, producing an antigen-antibody-HRP antibody "sandwich".

Then ELISA Substrate Solution is added, produces a blue color with its intensity proportional to the concentration of antibody from the sample bound to the antigen on plate. Finally, the Stop solution changes the reaction color from blue to yellow, and the microwell absorbance is read at 450 nm and 650 nm with a microplate reader.

## Materials to be Provided by the End-User

- Deionized (DI) water
- A microplate reader capable of measuring absorbance at 405 nm and 650 nm
- Adjustable pipettes to measure volumes ranging from 2 µL to 1 mL
- Software for data analysis (or Microsoft Excel, or Open office calc etc.)
- Tubes to prepare standard dilutions
- Timer
- Absorbent paper

## Storage Information

- Store the kit between 2°C and 8°C.
- After reconstitution of the lyophilized goat anti human IgG/A/M HRP, standard with 1X Assay Diluent, and store at -70°C for up to one month. Avoid repeated freeze/thaw cycles.
- Prior to use, bring all components to room temperature (18°C-25°C). Upon assay completion, return all components to appropriate storage conditions.

## Specimen Collection and Handling

***Serum:*** Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 X *g*. Remove serum layer and assay immediately or store serum samples at < -20°C. Avoid repeated freeze/thaw cycles. Serum specimens should be clear and non-hemolyzed.

***Plasma:*** Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1,000 X *g* within 30 minutes of collection. Assay immediately or store plasma samples at < -20°C. Avoid repeated freeze/thaw cycles. Plasma specimens should be clear and non-hemolyzed.

## Reagents Prepared Before Assay

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS provided by the supplier or manufacturer.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.
3. Re-constitute goat anti human IgG/A/M-HRP by adding 1 mL DI water to the vial, and dilute with 9 mL DI water.
4. Re-constitute RDRP antibody standards by adding 250 uL of DI water to each vial.
5. Dilution of Wash buffer, add 18 mL DI water per 2 mL wash buffer concentrate.
6. Samples: For cell culture supernatant samples, the end user may need to determine the dilution factors in a preliminary experiment. If dilution is required, samples should be diluted in 1X Assay Diluent before adding to the wells. It is recommended that serum, plasma be diluted 250 fold, 500 fold and 1000 fold in 1X Assay Diluent, measure three different levels.

## Assay Procedure

***Do not use sodium azide in any solutions, as it inhibits the activity of the horseradish-peroxidase enzyme.***

1. Add 100 µL/well of standards or samples or controls to the appropriate wells. If dilution is required, samples should be diluted in 1X Assay Diluent A before being added to the wells.
2. Seal plate and incubate at RT for 40 minutes with shaking.
3. Wash plate 4 times with 1x ELISA Wash Buffer.
4. Add 100 µL of diluted goat anti-human IgG/A/M HRP Antibody solution to each well, seal plate and incubate at RT for 40 minutes with shaking.
5. Wash plate 4 times with 1x ELISA Wash Buffer.
6. Add 100 µL of ELISA Substrate Solution and incubate **in the dark** for 15 minutes*. Positive wells should turn blue in color. It is not necessary to seal or shake the plate during this step.
7. Stop reaction by adding 100 µL of Stop Solution to each well. Positive wells should turn from blue to yellow.
8. Read absorbance at 405 nm within 15 minutes. If the reader can read at 650 nm, the absorbance at 650 nm can be subtracted from the absorbance at 405 nm.

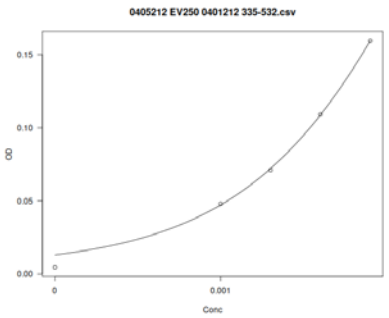
***Optimal substrate incubation time depends on laboratory conditions and the optical linear ranges of ELISA plate readers.**

## Calculation of Results

1. Export the data from ELISA reader software as .txt document.
2. Import to Microsoft Excel sheet, analyze the data with Excel plugin or R scripts, both plugin and R scripts are provided by the manufacturer upon request.
3. The titer is calculated by four-parameter log-logistic model.

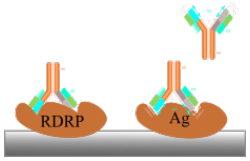
## Typical Data

***Fitting Curve:*** This fitting curve is generated at by four-parameter log-logistic model with each individual assay.

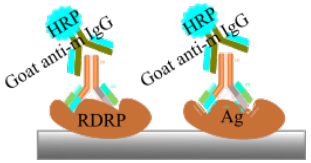



## Assay Procedure Summary

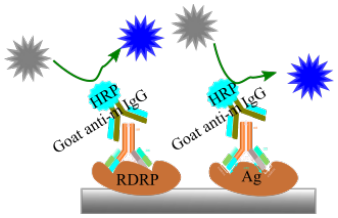
1. Add 100 µL diluted standards and samples, antibody binds to the antigens coated on plate (RDRP or other antigenic antigens) Incubate 2 hrs, RT, shaking




2. Wash 4 times, add 100 µL diluted goat anti human IgG/A/M Antibody Incubate 40 minutes at RT, shaking




3. Wash 4 times, add 100 µL HRP substrate, incubate 15 minutes at RT, avoid light
4. Add 100 uL of Stop solution, read absorbance at 405nm and 650nm as background.




5. Export the data as .txt file, analyze the results in Microsoft Excel or R scripts.

## Performance Characteristics

***Sensitivity:*** The minimum detectable concentration of polio virus antibody for this kit is 40 pg/mL (based on in house RDRP antibody cell culture supernatant estimation).

***Specificity:*** No cross reactivity was checked.

## Troubleshooting

High Background:

1. Background wells were contaminated.
2. Matrix used had endogenous antibodies.
3. Plate was insufficiently washed.
4. ELISA Substrate Solution was contaminated.

No signal:

5. Incorrect or no antibodies were added.
6. Goat anti human IgG/A/M HRP was not added.
7. Substrate solution was not added.
8. Wash buffer contains sodium azide.

Low or poor signal for the standard curve:

7. Standard was incomplete or was stored improperly.
8. Wash buffer contains sodium azide.

Low or poor signal for the standard curve:

9. Standard was incompletely reconstituted or was stored improperly.
10. Reagents were added to wells with incorrect concentrations.
11. Plate was incubated with improper temperature, timing, or agitation.

Signal too high, standard curves saturated:

13. Standard was reconstituted with less volume than required.
14. One or more reagent incubation steps were too long.
15. Plate was incubated with inappropriate temperature, timing, or agitation.

Sample readings out of range:

16. Samples contain no or below detectable levels of antibody.
17. Samples contain antibody concentrations greater than highest standard point.

High variations in samples and/or standards:

18. Pipetting errors may have occurred.
19. Plate washing was inadequate or no uniform.
20. Samples were not homogenous.
21. Samples or standard wells were contaminated.

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