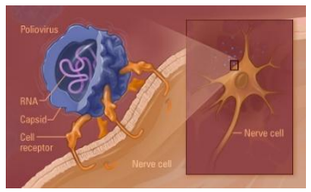
INTENDED USE

The Monkey Anti-Polio Virus 1-3 IgG ELISA Kit detects and quantifies polio type 1, 2 & 3 IgG in monkey serum or plasma of exposed or immunized animals. This immunoassay is suitable for:

- Determining immune status relative to non-immune controls
- Assessing efficacy of vaccines, including dosage, adjuvantcy, route of immunization, and timing
- Qualifying and/or standardizing vaccine batches and protocols

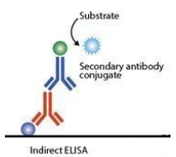
This kit is for research use only (RUO), not for diagnosis or therapeutic purposes.

INTRODUCTION



Poliomyelitis, often called polio or infantile paralysis, is an acute viral infectious disease caused by any of the three serotypes of polioviruses (PV1, PV-2, and PV-3). **Type 1** (Brunhilde): often with severe symptoms, **Type 2** (Lansing): with milder symptoms, **Type 3** (Leon): rare, but with severe symptoms. Antibodies to poliovirus can be diagnostic, and are generally detected in the blood of infected patients early in the course of infection. Two types of **vaccines** are used throughout the world to combat polio: 1) **Salk vaccine**, **or inactivated poliovirus vaccine** (**IPV**), is based on three wild, virulent reference strains, Mahoney (type 1), MEF-1 (type 2), and Saukett (type 3) polio viruses, grown in a type of monkey kidney tissue culture (Vero cell line), which are then inactivated with formalin; 2) **Oral polio vaccine** (**OPV** or Sabin's vaccine) is a **live-attenuated vaccine**, produced by the passage of the virus through non-human cells at a sub-physiological temperature, which produces spontaneous mutations in the viral genome.

PRINCIPLE OF THE TEST



The Monkey Anti-Polio Virus IgG ELISA kit is based on the binding of monkey anti-polio IgG in samples to polio immobilized on the microwells, and anti-polio IgG antibody is detected by anti-monkey IgG specific antibody conjugated to HRP (horseradish peroxidase) enzyme. After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the substrate, which is directly proportional to the amount of anti- polio IgG present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microwell reader. The activity of monkey IgG antibody in samples is calculated relative to anti-polio calibrators.

PRODUCT SPECIFICATIONS

Specificity
Purified recombinant capsid proteins (types 1, 2 &3) are used to coat the microwells; stabilizing postcoat contains BSA; thus, no other antibody specificity is detectable in the assay. The anti-Monkey IgG HRP conjugate specifically detects IgG, and will not react with IgM, IgA, or IgE class antibodies (see also page 7).

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8° C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

To Be Reconstituted: Store as indicated.

Component	Preparation Instructions
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at 4° C for long term and RT for short term.
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample/Conjugate Diluent** and store at 2-8° C until the kit lot expires or is used up.
Anti-Monkey IgG-HRP Conjugate Concentrate (100x) Part: H-MkG.2a11, 0.15ml	Peroxidase conjugated anti-monkey IgG in buffer with detergents and antimicrobial. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample/Conjugate Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8° C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
Polio Virus Coated Strip Plate	970101	8-well strips (12)	Coated with polio 1-3 capsid proteins; post-coated with stabilizers.
Anti- Polio IgG Calibrators			
3 U/ml 10 U/ml 30 U/ml 90 U/ml	970182B 970182C 970182D 970182E	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing anti- polio antibodies; in buffer with antimicrobial.
Anti- Polio Positive Control	970182 PC	0.65 ml	Anti-polio diluted in buffer with protein, detergents and antimicrobial. [Value range on label]
Low NSB Sample Diluent Reduces non-specific binding	TBTm **Not** for HRP Conjugate dilution	30 ml	Buffer with protein, detergents and antimicrobial. Use as is for sample dilution. See **Assay Design**, page 3.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml.
- Disposable glass or plastic 5-15ml tubes
- Stock bottle to store diluted Wash Solution; 0.2 to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- ELISA reader at 450 nm and ELISA plate washer

ASSAY DESIGN AND SET-UP

Sample Collection and Handling
Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

Antibody Stability & Dilution
Initial dilution of serum into **Working Sample Diluent** (WSD) is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for years, stored refrigerated or frozen. Further dilution into **Low NSB Sample Diluent** (LNSD), which provides the lowest assay background, should be at least 5 times the initial dilution and performed the same week as the assay.
Example: Initial (1/5): **10**ul serum + **40**ul WSD [or 0.1ml + 0.4ml]
Further (1/50): **10**ul initial (1/5) + **90**ul LNSD (1/50)

Assay Design
Review Interpretation of Results (p. 5-7) before proceeding:

- Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be lower than the **3 U/ml Calibrator**. This is usually 1:100 or greater dilution for monkey serum with normal levels of IgG and IgM.
- Run a Sample Diluent **Blank**. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required. Blank OD should be <0.3.
- Run the Anti-Polio Positive Control; the value range is on the label.
- Run a set of **Calibrators**, which validate that the assay was performed to specifications: **90 U/ml** should give a high signal (>1.5 OD); **3 U/ml** should give a low signal which can be used to discriminate at the Positive/Negative threshold (see Interpretation of Results, p. 5).

Plate Set-up
Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicates are recommended, including 8 Calibrator wells and 2 wells for each sample control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 1-5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of calibrators, samples, and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-Monkey IgG HRP to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 1.

3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

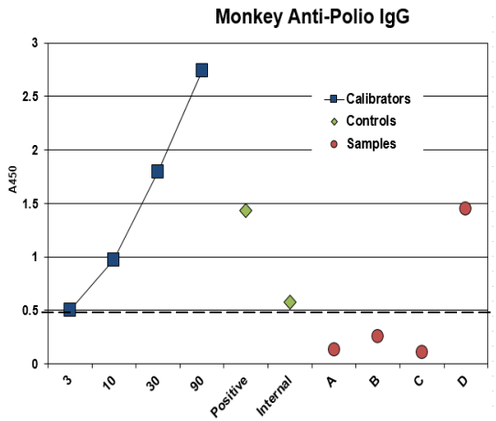
INTERPRETATION OF RESULTS

A. Antibody Activity Threshold Index

Compare Samples to **3 U/ml Calibrator** or **Internal Control**

= Positive/Negative Cut-off.

Example:



Results

The **sensitivity** of the assay to detect anti-polio IgG, from either natural infection or vaccination, is controlled so that the **3 U/ml Calibrator** represents a threshold OD for most true positives in monkey serum diluted to 1:100 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of antiserum from polio VP1 immunization, shows the OD range of the assay; high value indicates optimal sensitivity of the assay.

3 U/ml: a 'Cut-off' line has been drawn to indicate a threshold distinguishing between **Positive/Negative**. This is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives.

Positive Control – serum showing reactivity to polio VP1; the value range is on the label. This Control may be used to gauge precision and to normalize between-assay variation.

Internal Control – a true positive from an immune monkey that represents the investigator's experience in distinguishing low positive from negative samples (not in kit). This should be run in each assay to supplement the 3 U/ml Calibrator for Positive/Negative discrimination purposes.

Samples A,B,C,D – 3 samples (1:100) (A, B, C) are negative: below the threshold; 1 sample (D) is positive: clearly above the threshold.

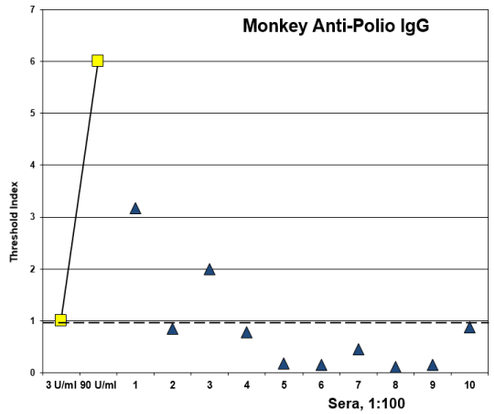
The 3 U/ml Calibrator can be used to calculate a **Threshold Index** that numerically discriminates Positive/Negative:

- Divide each Sample net OD by the 3 U/ml Calibrator net OD. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

INTERPRETATION OF RESULTS (cont)

Example:

Monkey Serum IgG
A panel of sera from non-immunized laboratory rhesus & cynomolgus monkeys was tested for anti-polio IgG (1:100 dilution in Low NSB Sample Diluent). **Threshold Index** was calculated using the **3** U/ml **Calibrator**.



Results

Anti-Polio Virus IgG:
Five (5) monkey sera were negative (below 1.0 TI); two sera (1,3) were positive (above 1.0 TI); three sera (2,4,10) were borderline.

Notes:

1. **Positives** may be due to prior encounter with the virus or from polio immunization.
2. When the **Positive Index** is **above 5.0,** using a dilution curve to calculate titer is a more accurate quantitation method (see Method C).
3. The **sensitivity** of the assay may be adjusted by changing the sample dilutions: a) increase dilution (e.g., 1:200) to lower the signals of borderline positives to negative; b) decrease dilution (e.g., 1:50) to convert borderline samples to positive. With the latter, the values of negatives may increase, so an alternative threshold should be considered using known negatives to develop a **Positive Index (**see below) or use an **Internal Control** (Page 5).

B. Positive Index
Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a **Positive Index**. One typical method is as follows:

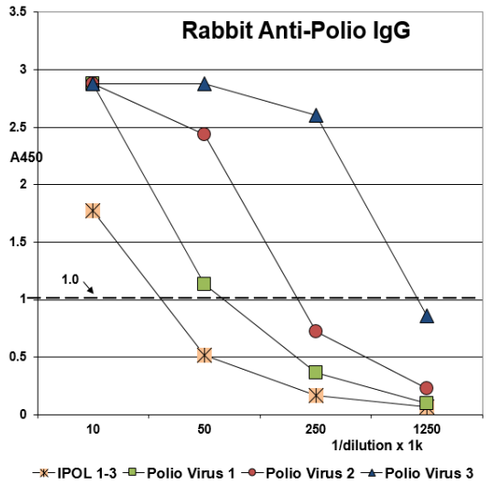
1. Calculate the net OD mean + 2 SD of the Control/Non-immune samples = **Positive Index**.
2. Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

INTERPRETATION OF RESULTS (cont)

A sample value would be **Positive** if significantly above the value of the pre-immune serum sample or a suitably determined non-immune panel or pool of samples, tested at the same sample dilution.

This calculation also **quantifies** the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

C. Antibody Titer
The most accurate method for comparing antibody potencies is by calculation of a titer, using an OD reading midrange in the dilution curves of each antibody as **Index.** In the example below, **IgG** titers were calculated as inverse of the dilution that produced a **1.0 OD** in the assay.



Results

IPOL 1-3: rabbit immunized with IPOL Poliovirus Vaccine [Sanofi Pasteur], 3 doses. Titer: **27k**.

Polio Virus 1: rabbit immunized with recombinant capsid protein Type 1, 6 doses with CFA. Titer: **66 k.**

Polio Virus 2: rabbit immunized with recombinant capsid protein Type 2, 6 doses with CFA. Titer: **190 k.**

Polio Virus 3: rabbit immunized with recombinant capsid protein Type 3, 6 doses with CFA. Titer: **1100 k.**

PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices and avoid ingestion or contact of any reagent with skin, eyes, or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid, and BND can be requested or obtained from the ADI website.

Instruction Manual No. M-970-150-PMG

Monkey Anti-Polio Virus 1-3 IgG ELISA Kit

Cat. #970-150-PMG, 96 tests

For Quantitation of Anti-Polio Virus 1-3 IgG in Serum, Plasma or other Biological Fluids

For research use only, not for diagnostic or therapeutic use.

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ELISA Kit Components	Amount	Part #
Polio 1-3 Antigens Coated Strip Plate	8-well strips (12)	970101
Anti-Polio IgG Positive Control	0.65 ml	970182PC
Anti-Polio IgG Calibrator 3 U/ml	0.65 ml	970182A
Anti-Polio IgG Calibrator 10 U/ml	0.65 ml	970182B
Anti-Polio IgG Calibrator 30 U/ml	0.65 ml	970182C
Anti-Polio IgG Calibrator 90 U/ml	0.65 ml	970182D
Anti-Monkey IgG HRP Conjugate (100X)	0.15 ml	H-MkG.2a11
Sample Diluent (20x)	10 ml	SD20T
Low NSB Sample Diluent	30 ml	TBTm
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-970-150-PMG