

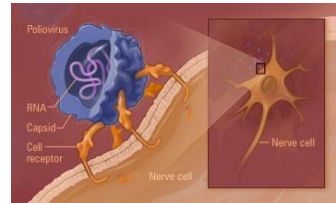
INTENDED USE

The **Rabbit Anti-Polio Virus 1-3 IgG** ELISA Kit detects and quantifies polio type 1, 2 & 3 IgG in rabbit 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

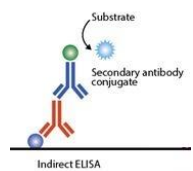


Poliomyelitis, often called polio or infantile paralysis, is an acute viral infectious disease caused by any of the three serotypes of polioviruses (PV1, PV-2, and PV-3).

Type 1 (Brunnhilde):

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 **Rabbit Anti-Polio Virus IgG** ELISA kit is based on the binding of rabbit anti-polio IgG in samples to polio immobilized on the microwells, and anti-polio IgG antibody is detected by anti-rabbit 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 rabbit 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-Rabbit 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-Rabbit IgG-HRP Conjugate Concentrate (100x) Part: H-RbG-211, 0.15ml	Peroxidase conjugated anti-rabbit 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	970182B	1.0ml	Four (4) vials, each containing anti- polio antibodies; in buffer with antimicrobial.
10 U/ml	970182C	1.0ml	
30 U/ml	970182D	1.0ml	
90 U/ml	970182E	1.0ml	
Anti- Polio Positive Control	970-182-PC	1.0ml	Anti-polio diluted in buffer with protein, detergents and antimicrobial. [Value range on label]
Low NSB Sample Diluent	TBTm	30 ml	Buffer with protein, detergents and antimicrobial. Use as is for sample dilution. See Assay Design, page 3.
Reduces non-specific binding	Not for HRP Conjugate dilution		
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): **10ul** serum + **40ul** WSD [or 0.1ml + 0.4ml]
Further (1/50): **10ul** initial (1/5) + **90ul** LNSD (1/50)

Assay Design

Review Interpretation of Results (p5-7) 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 rabbit 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 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.

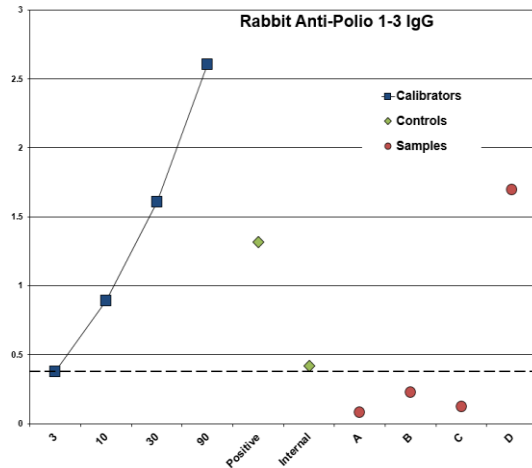
- 1st Incubation [100ul – 60 min; 4 washes]**
 - Add 100ul of calibrators, samples and controls each to pre-determined wells.
 - Tap the plate gently to mix reagents and incubate for 60 minutes.
 - Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.
 - 2nd Incubation [100ul – 30 min; 5 washes]**
 - Add 100ul of diluted Anti-Rabbit IgG HRP to each well.
 - Incubate for 30 minutes.
 - Wash wells 5 times as in step 2.
 - 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 rabbit 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 rabbit 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:

Rabbit Serum IgG

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

