**ELISA kits available from ADI (see details at the web site)**

| Catalog# | ProdDescription |
|---|---|
| 970-100-PHG | Human Anti-Poliomyelitis Viruses 1-3 (IPOL/IPV/OPV) IgG ELISA Kit, 96 tests, |
| 970-120-PMG | Mouse Anti-Poliomyelitis Viruses 1-3 (IPOL/IPV/OPV) IgG ELISA Kit, 96 tests, |
| 970-130-PRG | Rabbit Anti-Poliomyelitis Viruses 1-3 (IPOL/IPV/OPV) IgG ELISA Kit, 96 tests, |
| 970-140-PRM | Rabbit Anti-Poliomyelitis Viruses 1-3 (IPOL/IPV/OPV) IgM ELISA Kit, 96 tests, |
| 970-150-PMG | Monkey Anti-Poliomyelitis Viruses 1-3 (IPOL/IPV/OPV) IgG ELISA Kit, 96 tests, |
| 970-160-VPG | Mouse Anti-Poliomyelitis VP 1 (Sabin; POLV1-VP1) IgG ELISA Kit, |
| 970-165-VPG | Rabbit Anti-Poliomyelitis VP1 1 (Sabin; POLV1-VP1) IgG ELISA Kit, |
| 970-170-VPG | Human Anti-Poliomyelitis VP1 1 (Sabin; POLV1-VP1) IgG ELISA Kit |
| 970-130-PRG | Rat Anti-Poliomyelitis Viruses 1-3 (IPOL/IPV/OPV) IgG ELISA Kit, 96 tests, |
| | |
| POLV11-S | Anti-Poliomyelitis Viruses 1-3 (IPOL/IPV vaccine: Mahoney, MEF-1, and Saukett) antiserum |
| POLV12-M | Mouse monoclonal Anti-Poliomyelitis Virus 1-3 IgG, aff pure |
| POLV13-A | Anti-Poliomyelitis Virus 1-3 IgG |
| POLV13-BTN | Anti-Poliomyelitis Virus 1-3 IgG-Biotin Conjugate |
| POLV13-FITC | Anti-Poliomyelitis Virus 1-3 IgG-FITC Conjugate |
| POLV13-HRP | Anti-Poliomyelitis Virus 1-3 IgG-HRP Conjugate |
| POLV14-M | Mouse monoclonal Anti-Poliomyelitis Virus 1 IgG, aff pure |
| POLV15-C | Recombinant Poliomyelitis VP 1 (Sabin; POLV1-VP1) control for Western blot |
| POLV15-R-10 | Recombinant (E. Coli) Poliomyelitis VP 1 (Sabin; POLV1-VP1, 302-aa; full length, |
| POLV15-S | Anti-Poliomyelitis Virus 1 Viral Protein 1 (Sabin; POLV1-VP1) antiserum |
| POLV16-S | Anti-Poliomyelitis Virus 1 (LSc,2ab strain) antiserum, neutralizing |
| POLV21-M | Mouse monoclonal Anti-Poliomyelitis Virus 2 IgG, aff pure |
| POLV22-S | Anti-Poliomyelitis Virus 2 (P712,Ch,2ab strain) antiserum, neutralizing |
| POLV23-S | Anti-Poliomyelitis Virus 2 (sabin strain, native) antiserum, neutralizing |
| POLV31-M | Mouse monoclonal Anti-Poliomyelitis Virus 3 IgG, aff pure |
| POLV32-S | Anti-Poliomyelitis Virus 3 (Leon1,Ch,2ab strain) antiserum, neutralizing |
| POLV33-S | Anti-Poliomyelitis Virus 3 (sabin strain, native) antiserum, neutralizing |
| | |
| 4200 | Human Anti-Hepatitis B Surface Antigen (anti-HBsAg) IgG ELISA kit |
| 4205 | Human Anti-Hepatitis B Surface Antigen (anti-HBsAg) IgM ELISA kit |
| 510-100-HRG | Human Anti-Rubella Virus IgG ELISA kit |
| 510-110-HRM | Human Anti-Rubella Virus IgM ELISA kit |
| 520-100-HMG | Human Anti-Mumps Virus (parotitis) IgG ELISA, 96 tests, Quantitative |
| 520-110-HMM | Human Anti-Mumps Virus (parotitis) IgM ELISA, 96 tests, Quantitative |
| 520-200-HVG | Human Anti-Varicella Zoster Virus (chikenpox) IgG ELISA, 96 tests, Quantitative |
| 520-210-HVM | Human Anti-Varicella Zoster Virus (chikenpox) IgM ELISA, 96 tests, Quantitative |
| 530-100-HMG | Human Anti-Measles IgG ELISA kit, 96 tests |
| 530-110-HMM | Human Anti-Measles IgM ELISA kit, 96 tests |
| 600-020-HRV | Human Anti-Rabies Virus IgG ELISA Kit, 96 tests, Quantitative |
| 600-120-HRV | Human Anti-Rabies Virus Glycoprotein (RVG) IgG ELISA Kit, 2x 96 tests, |
| 600-220-HRV | Human Anti-Rabies Virus Nucleoprotein (RV-NP) IgG ELISA Kit, 2x 96 tests, |
| 600-300-100 | Human Anti-Meningococcal Group A Oligosaccharides-Diphtheria CRM197 IgG |
| 600-300-105 | Human Anti-Meningococcal Group CWY Oligosaccharides-Diphtheria CRM197 |
| 600-300-115 | Human Anti-Meningococcal Group ACWY Oligosaccharides-Diphtheria CRM197 |
| 900-160-83T | Human Anti-Anthrax Protective Antigen 83 (PA83) Ig's ELISA kit |
| 910-160-JEM | Human Anti-Japanese encephalitis virus (JEV) IgG specific ELISA kit |
| 910-170-JEM | Human Anti-Japanese encephalitis virus (JEV) IgM specific ELISA kit |
| 920-040-HAG | Human Anti-Influenza A virus IgG ELISA kit |
| 920-050-HAM | Human Anti-Influenza A virus IgM ELISA kit |
| 920-060-HAA | Human Anti-Influenza A virus IgA ELISA kit |
| 930-100-TTH | Human Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative |
| 940-200-DHG | Human Anti-CRM197 (Diphtheria Toxin mutant) IgG ELISA kit |
| 950-110-AHG | Human Anti-Adenovirus IgG ELISA kit |
| 950-120-AHM | Human Anti-Adenovirus IgM ELISA kit |
| 960-220-PHM | Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgM ELISA kit, |
| 960-250-PHG | Human Anti-B. pertussis Pertactin IgG ELISA kit |
| 980-100-PHG | Human Anti-H. Influenzae B (Hib) polyribosyl phosphate (PRP) IgG ELISA Kit, 96 |
| 980-110-PHM | Human Anti-H. Influenzae B (Hib) polyribosyl phosphate (PRP) IgM ELISA Kit, 96 |
| 990-100-THA | Human Anti-Mycobacterium Tuberculosis IgA ELISA kit, 96 tests |
| 990-110-THG | Human Anti-Mycobacterium Tuberculosis IgG ELISA kit, 96 tests |
| 990-120-THM | Human Anti-Mycobacterium Tuberculosis IgM ELISA kit, 96 tests |



*Instruction Manual No. M-970-130-PRG*

# Rabbit Anti-Poliomyelitis Virus 1-3 IgG ELISA KIT

## Cat. # 970-130-PRG; 96 Tests

For the detection of IgG antibody to Polio viruses 1-3 IgG in Rabbit serum or plasma or other biological fluids


*For research use only (RUO), not for diagnosis, cure or prevention of the disease.*

ALPHA DIAGNOSTIC INTERNATIONAL

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.
Phone (210) 561-9515 • Fax (210) 561-9544
Toll Free (800) 786-5777

Email: service@4adi.com

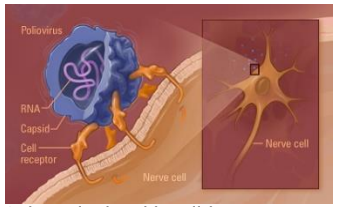
Web Site: www.4adi.com

| Kit Components (96 tests) | |
|---|---|
| Polio virus antigens 1-3 antigen coated strip plate, (8x12 strip or 96 wells) # 970-101 | 1 plate |
| **Anti-polio IgG Std A** (1 mL; Polio virus IgG 3 U/ml) #970132A | 1 vial |
| **Anti-polio IgG Std B** (1 mL; Polio virus IgG 10 U/ml) #970132B | 1 vial |
| **Anti-polio IgG Std C** (1 mL; Polio virus IgG 30 U/ml) #970132C | 1 vial |
| **Anti-polio IgG Std D** (1 mL; Polio virus IgG 90 U/ml) #970132D | 1 vial |
| Anti-Rabbit IgG-HRP **Conjugate**, (0.15 ml, 100X) #970133 | 1 bottle |
| **Sample Diluent (SD20T, 20X**, 10 ml # SD-20T | 1 bottle |
| **Wash buffer** (10X) 60 ml # 970130-WB | 1 bottle |
| **TMB Substrate** Solution, 15 ml #970130-TMB | 1 bottle |
| **Stop Solution**, 15 ml # 970130-ST | 1 bottle |
| Complete Instruction Manual | 1 |

## Intended Use

ADI **Polio viruses 1-3 IgG** ELISA Kit is intended for the detection of IgG antibody to Polio virus in Rabbit serum, plasma or other biological fluids. The kit is well suited for testing human polio vaccine formulations in Rabbit. The kit contains no active virus or bacteria. For research use only (RUO), not for diagnosis, cure or prevention of the disease.

## Introduction




Poliomyelitis, often called polio or infantile paralysis, is an acute viral infectious disease spread from person to person, primarily via the fecal-oral route. Different types of paralysis may occur, depending on the nerves involved. The term poliomyelitis is used to identify the 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. The virus enters the central nervous system in about 3% of infections. Most patients with CNS involvement develop non-paralytic aseptic meningitis, with symptoms of headache, neck, back, abdominal and extremity pain, fever, vomiting, lethargy and irritability. A laboratory diagnosis is usually made based on recovery of poliovirus from a stool sample or a swab of the pharynx. 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. The first is **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. **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. This vaccine is unable to replicate in the brain.

**IPOL**®, Poliovirus Vaccine Inactivated, produced by Sanofi, contains three types of polioviruses: **Type 1 (Mahoney), Type 2 (MEF-1), and Type 3 (Saukett)**. IPOL vaccine is



### QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:
1. The O.D. of the blanks should be < 0.500.
2. The A450 value of the highest std (90 U/ml0 should be >1.00.

## Rabbit sample testing

A population of non-vaccinated, healthy adult Rabbits was tested for the basal level of the antibodies to polio antigens by ELISA at a serum dilution of 1:100. Most samples showed A450 values of <0.500. This should be considered base value or background values. Animals showing significantly higher values should be considered positive or an induction of antibodies due to vaccination. Users must establish their reference values for their strains, sex, age, and exposure to the polio virus or vaccines.

| **Samples** | Blank | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 |
|---|---|---|---|---|---|---|---|---|---|
| **A450** | 0.323 | 0.466 | 0.48 | 0.451 | 0.395 | 0.320 | 0.42 | 0.43 | 0.38 |

### INTERPRETATION

There are no recommended guidelines for polio antibodies in Rabbit. Each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations, exposure to the virus or vaccination.

### Polio Vaccine Testing in Animals & Humans

Monkey can be experimentally infected with polio virus and used for research. For vaccine use, polio virus is grown monkey derived VERO cells in culture. Polio strains have been developed that can replicate in mouse and rat as well. Rats are also used to discover novel methods of vaccinations such as 'Nanopatch or microneedles".

Human samples obtained from vaccinated individuals tested positive using the polio virus antigen coated used in this kit. Rabbits and mice vaccinated with Ipol, OPV/IPOV vaccine also produced high tittered antibodies to the antigens used in the kit.

ADI also cloned and expressed Polio virus 1 (Sabin) viral protein 1 (Polv1-VP1, Cat# POLV15-R-10) and vaccinated mouse and rabbits. The resulting antiserum reacted with the human polio viruses 1-3 antigens used in this test. Human samples obtained from polio-vaccinated individuals also reacted with the recombinant POLV-VP1. Therefore, POLV1-VP1 is highly represented in the antigen mix used in the kit, and the anti-VP1 reacting with the natural polio viruses 1-3 VP1s.

### Specificity

Polio viruses' 1-3 antigens are used in the kit. Therefore, antibodies to all three polio serotype will be detected in this test. Anti-Rabbit IgG-HRP conjugate has been optimized to detect all IgG but not the IgM or IgA.

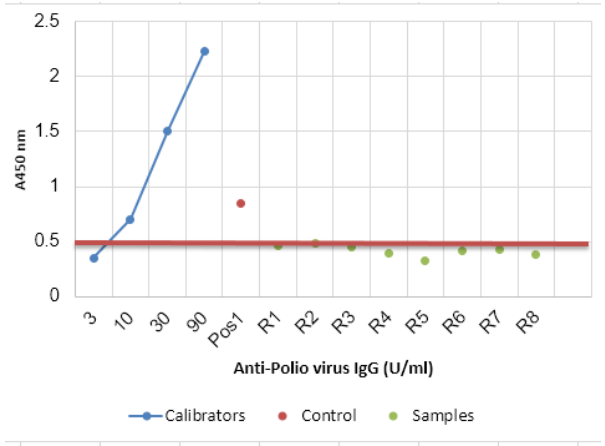
### References:

Pinheiro FP et al; World Health Stat Q 50(3/4):161-169, 1997; Gubler DJ et al; Infect Agents Dis 2:383-393, 1993; Wu SJ et al Clin Diagn Lab Immunol1997; 4(4):452-7; Lam SK; Clin Diagn Virol 1998;10(1):75-8; Rossi CA; 1995-1996. Am J Trop Med Hyg 1998;59(2):275-8 2008-12-18

## WORKSHEET OF A TYPICAL ASSAY

NOTE: These data are for demonstration purpose only. It must not be used to determine the sample results.

| Stds | U/ml | Average A450 | Net A450 |
|---|---|---|---|
| Sample diluent (blank) | 0 | 0.300 | - |
| Rabbit Anti-Polio IgG | 3 | 0.65 | 0.35 |
| Rabbit Anti-Polio IgG | 10 | 1.0 | 0.70 |
| Rabbit Anti-Polio IgG | 30 | 1.81 | 1.51 |
| Rabbit Anti-Polio IgG | 90 | 2.53 | 2.23 |
| Rabbit Sample 1 | | 0.48 | 0.18 |




*/2-Nas/970-130-PRG-ELISA-graph

Typical Std Curve (do not use this for sample calculation)

## CALCULATION OF RESULTS

The mean values for the measured absorptions are calculated after subtraction of the blank values from the controls and standards.

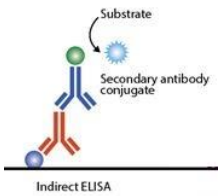
The OD of the calibrators (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 parameter logistics or Logit-Log. The initial dilution of unknowns (1:100) has been taken into consideration when reading the results from the graph. Therefore, do not multiply the sample values if used at 1:100 dilutions. Results of unknowns of higher predilution (e.g., 1:500 have to be adjusted for the dilution factor and multiplied by 5). Unknowns showing concentrations above the highest std have to be diluted as described in “Test Procedure” and reassayed.

All samples above the cut-of values may be considered positive for the presence of anti-polio virus IgG.

replicated in Vero cells. Each dose (0.5 mL) of trivalent vaccine is formulated to contain 40 D antigen units of Type 1, 8 D antigen units of Type 2, and 32 D antigen units of Type 3 poliovirus. Poliovirus Vaccine induces the production of neutralizing antibodies against each type of virus which are related to protective efficacy. There are 57 nucleotide substitutions which distinguish the attenuated Sabin 1 strain from its virulent parent (the Mahoney serotype), two nucleotide substitutions attenuate the Sabin 2 strain, and 10 substitutions are involved in attenuating the Sabin 3 strain. The primary attenuating factor common to all three Sabin vaccines is a mutation located in the virus's internal ribosome entry site (IRES) which alters stem-loop structures, and reduces the ability of poliovirus to translate its RNA template within the host cell. The attenuated poliovirus in the Sabin vaccine replicates very efficiently in the gut, the primary site of infection and replication, but is unable to replicate efficiently within nervous system tissue.

ADI's human anti-polio virus IgG ELISA kit detects antibodies to the three subtypes of polio viruses that are currently used in vaccines. This serological tests will determine IgG to polio viruses 1-3 that can be due to past illness or by vaccination.

## PRINCIPLE OF THE TEST




ADI's Polio virus IgG ELISA Kit is based on the principle of the enzyme immunoassay (EIA). Diluted patient serum is added to wells coated with purified Polio virus antigen. Polio virus IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme that produced blue color. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgG antibodies is directly proportional to the intensity of the color.

## MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5µl, 100µl, 500µl) and multichannel pipet with disposable plastic tips. Bidistilled water, reagent troughs, Orbital shaker, plate washer (recommended) and ELISA plate Reader (450nm).

## PRECAUTIONS

Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed. All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly. All reagents have to be brought to room temperature (18 to 25 °C) before performing the test. Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided. It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions. When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time. In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used. No reagents from different kit lots have to be used, they should not be mixed among one another. All reagents have to be used within the expiry period. In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation. The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.



Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).
http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

## SPECIMEN COLLECTION AND HANDLING

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 48 hours, for a longer storage they should be kept at -20 °C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.

Rabbit samples (not the standards) have to be diluted 1:100 with ready-to-use sample diluent (e.g. 5 µL serum + 495 µL sample diluent). Prepare at least 250-300 ul of diluted samples for testing. Do not store diluted samples beyond the assay date.

## REAGENTS PREPARATION

1. **Dilute Wash buffer** 1:10 with water. (**Dilute 60 ml stock with 540 ml distilled water**) Store diluted buffer at 4oC for 1 month. (If during the cold storage crystals precipitate, the concentrate should be warmed up at 37 degrees C for 15 minutes.

2. **Sample Diluent (Dilute 1:20 with water** (1 ml stock and 19 ml distilled water). Use 1x diluent for the dilution of the sample and to dilute the antibody-HRP Conjugate. Store at 2-4oC.

3. **Antibody-HRP Conjugate-**Stock is provided as 100x. Dilute 1:100 with 1X sample diluent (10 ul stock in 990 ul 1x sample diluent). Prepare 1 ml for each strip or 10 ml for full plate. Store at 2-4oC until used. Do not store diluted antibody conjugate beyond the assay date.

***All reagents must be at room temperature prior to their use.***

## STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8$^{o}$C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. The unused portions of the standards should be stored at 2-8$^{o}$C or stored frozen in small aliquots and should be stable for 3 months.

**TEST PROCEDURE** *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. Dilute all samples 1:100 with the sample diluent. It is recommended to prepare a parallel replica plates containing all sample for quick transfer to the coated plate. DO NOT dilute calibrators or controls. **Dilute wash buffer stock (10X) 1:10 with distilled water.**

1. Label or mark the microtiter well strips to be used on the plate
2. Dispense **100 ul** diluent in 1 well to be used as blank. Pipet **100 ul of** calibrators, controls, and diluted samples into appropriate wells in *duplicate*. See worksheet of a typical set-up on page 5. Cover the plate, mix gently for 5-seconds and incubate at room temp (25-28oC) for 60 min.

3. Aspirate the well contents and blot the plate on absorbent paper. Immediately, **wash the wells 3 times** with 300 ul of 1X wash buffer. We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

4. Add **100 ul antibody-HRP conjugate** to all wells leaving one empty for the substrate blank. Mix gently for 5-10 seconds. Cover the plate and incubate for **30 minutes** at room temp (18-26oC).

5. Wash the wells 4 times as in step 3.

6. Add **100 ul TMB substrate solution**. Mix gently for 5-10 seconds. Cover the plate and incubate for **20 minutes** at room temp. **Blue color** develops in positive controls and samples.

7. Stop the reaction by adding **100 ul of stop solution** to all wells. Mix gently for 5-10 seconds to have uniform color distribution (blue color turns yellow).

8. Measure the **absorbance at 450 nm** using an ELISA reader within 60 min.

**NOTES**

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at $4^{o}$C. Do not touch the bottom of the wells.