

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

950-100-AHA Human Anti-Adenovirus IgA ELISA kit, 96 tests, Quantitative
950-130-AMG Human Anti-Adenovirus IgG ELISA kit, 96 tests, Quantitative
950-120-AHM Human Anti-Adenovirus IgM ELISA kit, 96 tests, Quantitative

AE-320500-1 Mouse Anti-Zaire Ebola virus Nucleoprotein (NP) IgG ELISA Kit, 96 tests, Quantitative
AE-320510-1 Mouse Anti-Zaire Ebola virus Nucleoprotein (NP) IgM ELISA Kit, 96 tests, Quantitative
AE-320520-1 Human Anti-Zaire Ebola virus Nucleoprotein (NP) IgG ELISA Kit, 96 tests, Quantitative
AE-320530-1 Human Anti-Zaire Ebola virus Nucleoprotein (NP) IgM ELISA Kit, 96 tests, Quantitative
AE-320540-1 Rabbit Anti-Zaire Ebola virus Nucleoprotein (NP) IgG ELISA Kit, 96 tests, Quantitative
AE-320550-1 Monkey/Chimp Anti-Zaire Ebola virus Nucleoprotein (NP) IgG ELISA Kit, 96 tests, Quantitative
AE-320560-1 Monkey/Chimp Anti-Zaire Ebola virus Nucleoprotein (NP) IgM ELISA Kit, 96 tests, Quantitative

AE-320600-1 Mouse Anti-Zaire Ebola virus glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-320610-1 Mouse Anti-Zaire Ebola virus glycoprotein (GP) IgM ELISA Kit, 96 tests, Quantitative
AE-320620-1 Human Anti-Zaire Ebola virus glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-320630-1 Human Anti-Zaire Ebola virus glycoprotein (GP) IgM ELISA Kit, 96 tests, Quantitative
AE-320640-1 Rabbit Anti-Zaire Ebola virus glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-320650-1 Monkey/Chimp Anti-Zaire Ebola virus glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-320650-PC Monkey/Chimp Anti-Zaire Ebola virus glycoprotein (GP) IgG positive control
AE-320660-1 Monkey/Chimp Anti-Zaire Ebola virus glycoprotein (GP) IgM ELISA Kit, 96 tests, Quantitative
AE-320670-1 Dog Anti-Zaire Ebola virus glycoprotein IgG ELISA Kit, 96 tests, Quantitative
AE-320680-1 Pig Anti-Zaire Ebola virus glycoprotein IgG ELISA Kit, 96 tests, Quantitative

AE-320700-1 Mouse Anti-Zaire Ebola virus VP40 IgG ELISA Kit, 96 tests, Quantitative
AE-320710-1 Mouse Anti-Zaire Ebola virus VP40 IgM ELISA Kit, 96 tests, Quantitative
AE-320720-1 Human Anti-Zaire Ebola virus VP40 IgG ELISA Kit, 96 tests, Quantitative
AE-320730-1 Human Anti-Zaire Ebola virus VP40 IgM ELISA Kit, 96 tests, Quantitative
AE-320740-1 Rabbit Anti-Zaire Ebola virus VP40 IgG ELISA Kit, 96 tests, Quantitative
AE-320750-1 Monkey/Chimp Anti-Zaire Ebola virus VP40 IgG ELISA Kit, 96 tests, Quantitative
AE-320760-1 Monkey/Chimp Anti-Zaire Ebola virus VP40 IgM ELISA Kit, 96 tests, Quantitative

AE-320800-1 Zaire Ebola Virus Glycoprotein (EBOV GP antigen) ELISA Kit, 48 tests, Quantitative
AE-320800-96 Zaire Ebola Virus Glycoprotein (EBOV GP antigen) ELISA Kit, 96 tests, Quantitative
AE-320805-RT-10 Zaire Ebola Virus antigen (GP) rapid test (visual results in 2-10 mins), 10 cassettes/pk
AE-320810-1 Humanized (plant expressed) Anti-Ebola GP IgGs ELISA kit, 96 tests, Quantitative
AE-320815-1 Anti-Humanized Ebola GP IgGs (Plant expressed) (Anti-drug antibody/ADA) ELISA kit, 96 tests,
AE-320880-RT-25 Humanized Ebola IgG (humanized IgGs expressed in tobacco or other plants) rapid test
(results in 2-10 min), 25 cassettes/pk

AE-321600-1 Mouse Anti-Sudan Ebola virus glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-321610-1 Mouse Anti-Sudan Ebola virus glycoprotein (GP) IgM ELISA Kit, 96 tests, Quantitative
AE-321620-1 Human Anti-Sudan Ebola virus glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-321630-1 Human Anti-Sudan Ebola virus glycoprotein (GP) IgM ELISA Kit, 96 tests, Quantitative
AE-321640-1 Rabbit Anti-Sudan-Ebola virus glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-321650-1 Monkey/Chimp Anti-Sudan Ebola virus glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-321660-1 Monkey/Chimp Anti-Sudan Ebola virus glycoprotein (GP) IgM ELISA Kit, 96 tests, Quantitative

AE-322600-1 Mouse Anti-Marburg (Angola) virus glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-322610-1 Mouse Anti-Marburg (Angola) glycoprotein (GP) IgM ELISA Kit, 96 tests, Quantitative
AE-322620-1 Human Anti-Marburg (Angola) glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-322630-1 Human Anti-Marburg (Angola) glycoprotein (GP) IgM ELISA Kit, 96 tests, Quantitative
AE-322640-1 Rabbit Anti-Marburg (Angola) glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-322650-1 Monkey/Chimp Anti-Marburg (Angola) glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative
AE-322660-1 Monkey/Chimp Anti-Marburg (Angola) glycoprotein (GP) IgM ELISA Kit, 96 tests, Quantitative

AE-323620-1 Human Anti-Reston Ebola virus glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative

AE-324620-1 Human Anti-Bundibugyo virus glycoprotein (GP) IgG ELISA Kit, 96 tests, Quantitative

AE-325600-XH Human Anti-Zaire+Sudan+Reston+ Bundibugyo Glycoproteins combo IgG ELISA Kit, 96 tests,
AE-325600-XM Monkey Anti-Zaire+Sudan+Reston+ Bundibugyo Glycoproteins combo IgG ELISA Kit, 96

AE-327100-1 Mouse Anti-Adenovirus hexon antibody (hAdV Hxn) IgG ELISA Kit, 96 tests, Quantitative
AE-327110-1 Human Anti-Adenovirus hexon antibody (hAdV Hxn) IgG ELISA Kit, 96 tests, Quantitative
AE-327120-1 Monkey/Chimp Anti-Adenovirus hexon antibody (hAdV Hxn) IgG ELISA Kit, 96 tests, Quantitative
AE-327200-1 Mouse Anti-VSV Indiana Matrix (M) antibody (VSVIM) IgG ELISA Kit, 96 tests, Quantitative
AE-327210-1 Human Anti-VSV Indiana Matrix (M) antibody (VSVIM) IgG ELISA Kit, 96 tests, Quantitative
AE-327300-1 Mouse Anti-VSV Indiana Matrix (M) antibody (VSVIM) IgG ELISA Kit, 96 tests, Quantitative
AE-327310-1 Human Anti-VSV Indiana Glycoprotein (GP) antibody (VSVIG) IgG ELISA Kit, 96 tests, Quantitative
AE-327320-1 Monkey/Chimp Anti-VSV Indiana Glycoprotein (GP) antibody (VSVIG) IgG ELISA Kit, 96 tests, Quantitative

Instruction Manual No. M-950-130-AMG

Mouse Anti-Human Adenovirus (Hxn) IgG

ELISA KIT Cat. # 950-130-AMG

For Detecting Mouse IgG antibodies against human Adenovirus (hxn) in Serum or Plasma

For In Vitro Research Use Only



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INTERNATIONAL**

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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHANGES.

Mouse Anti-Human Adenovirus IgG ELISA KIT Cat. # 950-130-AMG (96 tests)

Kit Components (96 tests)	Cat #
Adenovirus antigen coated strip plate, (8x12 strip or 96 wells) # 950131	1 plate
Anti-Human Adenovirus IgG (1 U/ml) 2 ml #95132A	1 vial
Anti-Human Adenovirus IgG (10 U/ml) 2 ml #950132B	1 vial
Anti-Human Adenovirus IgG (35 U/ml) 2 ml #950132C	1 vial
Anti-Human Adenovirus IgG (150 U/ml) 2 ml #950132D	1 vial
Anti-Mouse IgG-HRP Conjugate, (15 ml) #950133	1 bottle
Sample Diluent, 60 ml #950130-SD	1 bottle
Wash buffer (10X) 60 ml # 950130-WB	1 bottle
TMB Substrate Solution, 15 ml #950130-TM	1 bottle
Stop Solution, 15 ml # 950130-ST	1 bottle
Plastic foil for covering plate and Resealable bag for the un-used antigen strips	1
Complete Instruction Manual # M-950-130-AMG	1

Intended Use

Mouse Anti-Human Adenovirus IgG ELISA Test Kit has been designed for the detection of specific IgG class antibodies against human Adenovirus in serum and plasma. 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 standardizing vaccine batches & protocols.

The antigen used in the kit is human Adenovirus 5 (hexon) protein that is highly conserved within the human Adenovirus serotypes 1-57 (75%-90%). There is known preexisting immunity in humans to many serotypes and many have cross-reactive and neutralizing antibodies. The assay is for research use only (RUO) and is not intended diagnostic or therapeutic use.

Introduction

Adenoviruses (members of the family Adenoviridae) are medium-sized (90–100 nm), nonenveloped viruses with an icosahedral nucleocapsid containing a dsDNA genome. The adenovirus is a ubiquitous pathogen of humans and animals. Adenoviruses are also known to cause respiratory infections in horses, cattle, pigs, sheep, and goats. Adenoviruses have a broad range of vertebrate hosts; there are 57 accepted human adenovirus types (HAdV-1 to 57) in seven species (**Human adenovirus A to G**; Genus Mastadenovirus (including all human adenoviruses); type species: Human adenovirus C) have been found to cause a wide range of illnesses, from mild respiratory infections in young children to life-threatening multi-organ disease in people with a weakened immune system. Adenoviruses are endemic in all populations throughout the year. The infection is spread both through the aerial-droplet route and the routes characteristic for intestinal infections. Adenoviruses mainly infest respiratory and intestinal mucosa, but also the cornea. They are accumulated in the epithelial cells and regional lymph nodes. Adenoviruses cause the widest variety of illnesses of the known respiratory viruses. The adenovirus infection is the most frequently caused viral disease of the respiratory tract among preschool children (types 1 - 5 and 7). Pneumonia is the most severe form of adenoviral infection occurring mostly in infants below the age of one.

For **mouse samples**, we suggest that the users define their own limits of vaccinated or infected animals. A limited study of mouse samples (non-vaccinated, non-infected, and infected) for adenovirus IgG using this ELISA kit produced the following results

Samples	Healthy animals Non-vaccinated	Naturally infected
1:100 dilution	<0.10-0.20 ((n=10)	0.6-2.1 (n=3)

Quality Control

The test results are only valid if the test has been performed following the instructions. All standards and kit controls must be found within the acceptable ranges as stated on the vials. If criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. In case of any deviation the following technical issues should be proven (reagents, protocol, equipments, etc).

PERFORMANCE CHARACTERISTICS

Intra-Assay-Precision 8.1 %

Inter-Assay-Precision 11.3 %

Analytical Sensitivity ~1 U/mL

Interferences

No interferences to bilirubin up to 0.3 mg/mL; Hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL.

Cross Reactivity

No cross reactivity to Influenza A and RSV.

Specificity and Species Reactivity

The antigen used in the kit is human Adenovirus 5 (hexon) protein that is highly conserved within the human Adenovirus serotypes 1-57 (75%-90%) and within the various Adenovirus groups (A-D). There is known preexisting immunity in humans to many serotypes and many have cross-reactive and neutralizing antibodies. Therefore, it is expected that the kit may detect adenovirus antibodies from many serotypes.

Some **Ebola vaccines** (VRC207) have used chimp Ad3 vectors to deliver Ebola GP. The human Ad5 Hexon is 87% conserved in chAd3. It is expected that the chAd3 antibodies be also detected in this kit.

This kit detects only the mouse IgG isotype adenovirus antibodies and not the IgM or IgA. ADI has separate ELISA kits to detect IgM and IgA antibodies.

This kit is especially suited to detect and measure Adenovirus antibodies as a results of adenovirus-vectored vaccines. It is not intended to detect mouse adenovirus infections. ADI has separate ELISA kits that detect mouse adenovirus specific antibodies in animals.

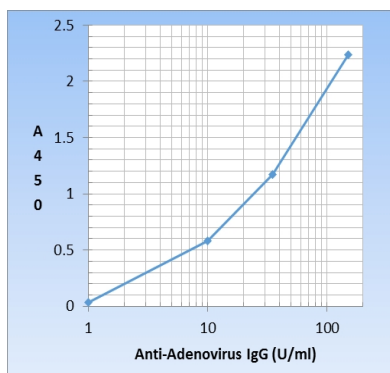
This kit is tested in mouse samples only. It has not been tested in rat, chickens or other animals. For human studies, we have separate ELISA kits.

References: Hierholzer, JC et al (1986) Adenoviruses, 527; McMinn PC, et al (1991) Community Outbreak Keratoconjunctivitis in due to Adenovirus 164;1113; Wadell G., et al (1988) Adenoviridae: The adenoviruses Vol 2: 284; Wigand, R. et al (1982) 18; 169.

WORKSHEET OF A TYPICAL ASSAY

Wells	Stds/samples	Mean A450	Net A450	Results
A1, A2	Blanks	0.100	-	-
B1, B2	Anti-Adenovirus IgG (1 U/ml)	0.138	0.038	
C1, C2	Anti-Adenovirus IgG (10 U/ml)	0.681	0.581	
D1, D2	Anti-Adenovirus IgG (35 U/ml)	1.269	1.169	
D1, D2	Anti-Adenovirus IgG (150 U/ml)	2.336	2.236	
E1, E2	Sample 1	0.915	0.90	36 U/ml

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



Arif/3_ADI_ELISA

CALCULATION OF RESULTS:

The obtained OD of the standards (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. For the calculation of the standard curve apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used). The concentration of the samples can be read from the standards curve. The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution have to be multiplied with the dilution factor. Samples showing concentrations above the highest standard have to be diluted as described in "Assay Procedure" and reassayed.

Interpretation of Results

The information is- based upon human samples.

Equivocal 8-10 U/ml

Positive Samples >10 U/ml

Negative samples: For a value below the cut-off standard (arbitrary units =10 U/ml) the sample should be interpreted as a negative result.

All samples with values above the Cut-off are considered positive. Positive control values must be at least twice the values of the Cut-off control for the test to be valid. Approx. 95% of normal population is positive for adenovirus IgG.

An acute adenoviral infection can be detected by virus isolation and/or serology. The infection is necessary to document infection. IgG is the predominant antibody class measured in the serologic tests.

Adenoviruses represent the largest nonenveloped viruses. They are able to be transported through the endosome (i.e., envelope fusion is not necessary). The virion also has a unique "spike" or fiber associated with each penton base of the capsid that aids in attachment to the host cell via the receptor on the surface of the host cell. Adenoviruses have long been a popular viral vector for gene therapy due to their ability to affect both replicating and non-replicating cells, accommodate large transgenes, and code for proteins without integrating into the host cell genome. Replication-deficient human adenovirus type 5 (Ad5) can be produced to high titers in complementing cell lines, such as PER.C6, and is widely used as a vaccine and gene therapy vector. However, preexisting immunity (neutralizing antibodies, NA) against Ad5 hampers consistency of gene transfer, immunological responses, and vector-mediated toxicities. Strategies to bypass NA to Ad5 viruses include switching of adenovirus type and use of animal adenoviruses. Of the 47 types tested, subgroup B viruses Ad35 and Ad11 proved rarely neutralized by human sera. **Ebola Vaccine:** VRC 207 is a phase 1 clinical trial designed to determine the safety, side-effect profile, and immunogenicity of an investigational recombinant cAd3 ebolavirus vaccine (GP from the Zaire and Sudan strains as they are responsible for majority of Ebola cases). The vaccine was developed by Okairios (now owned by GSK), and demonstrated protection in NHP model cAd3 was selected as a vector due to low prevalence of preexisting Ad3 antibodies.

Adenovirus vectors are being tested for many vaccine for HIV, Hepatitis and Flu etc.

PRINCIPLE OF THE TEST

Alpha Diagnostic's Adenovirus IgG Antibody ELISA Test Kit is based on the principle of the enzyme immunoassay (EIA). Adenovirus antigen is bound on the surface of the microtiter strips. Diluted patient serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized Adenovirus antigen takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-Mouse-IgG peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. 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.

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. Nevertheless precautions like the use of latex gloves have to be taken. 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.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI 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. For the performance of the test the samples (not the standards) have to be diluted 1:101 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent).

Mouse Sample Dilution

We recommend testing for normal or non-immunized samples at 1:100 dilution. However, vaccinated samples be tested at several dilutions (1:500, 1:2000 etc) to determine the antibody levels that are within the detection range of the kit.

REAGENTS PREPARATION

1. **Dilute Wash buffer** 1:10 with water, (**60 ml stock in 540 ml distilled water**)
Store diluted buffer at 4°C for 1 month.

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°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°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).

Important: If you have not used this kit before, we recommend to use 1 or 2 strips to run the standards alone to get familiar with the test and not run the risk of making mistakes and lose sample or the whole kit.

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. **All samples should be diluted 1:100 (5 µl samples in 500 µl sample diluent).** Dilute the wash buffer with water (1:10). It is recommended to prepare a parallel replica plates containing all sample for quick transfer to the coated plate.

1. Label or mark the microtiter well strips to be used on the plate.
2. Dispense **100 µl diluent** in 1 well to be used as blank. Pipet **100 µl of Prediluted calibrators, controls, and samples** (diluted 1:100) 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 for 60 min.**
3. Aspirate the well contents and blot the plate on absorbent paper. Immediately, **wash the wells 4 times** with 300 µl 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 µl Mouse anti-IgG-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 (25-28°C).
5. **Wash the wells 3 times** as in step 3.
6. Add **100 µl 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 µl 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°C. Do not touch the bottom of the wells.