MICROGEN BIOPRODUCTS

CE

REF M481 ADENOSCREEN® EIA

96

IVD

INTENDED USE

Adenoscreen® EIA is an enzyme immunoassay for the detection of adenovirus antigen in human faecal samples. The kit is intended for professional laboratory use only.

PRINCIPLE OF THE TEST

Microtitration wells are coated with rabbit antibodies raised against adenovirus hexon antigen, a highly conserved common component of all adenoviruses. The diluted faecal samples are pipetted directly into the coated wells. Adenoviruses present in the sample bind specifically to the antibodies on the well. Antibody-enzyme conjugate is added to the wells containing samples and is co-incubated to permit binding to adenoviruses which have been captured by the coated antibody. Unbound sample and conjugate are rinsed away. Surface-bound, enzyme-labelled complex is then measured by reaction with the chromogen, tetramethyl benzidine (TMB). The intensity of the colour produced is proportional to the amount of adenovirus present in the original sample.

CONT	**KIT PRESENTATION**	
MT PLATE	M481a	1x96 well

Microwell Plate: 12 x 8 breakaway microwell strips coated with rabbit antibodies to adenovirus hexon antigen, sealed in a foil pouch with desiccant.

CONTROL	**+**	M481b	1.0mL

Positive Control (**Black** Cap). Dilute suspension of inactivated adenovirus antigen. Preserved with 0.01% thiomersal.

CONJ	M481c	5.0mL

Conjugate (**Red** Cap). Rabbit anti-adenovirus hexon antibody conjugated with horseradish peroxidase. Preserved with non-mercury based preservative.

BUF	**WASH**	**20x**	M480e	100mL

Wash Buffer. 20 x concentrate. Phosphate buffered saline pH 7.0-7.2 containing 0.05% Tween 20 at working strength.

SUBS	**TMB**	M480g	2 x 5mL

Substrate (**Blue** cap). TMB/peroxide.

SOLN	**STOP**	M480h	2 x 5mL

Stop Solution (**Yellow** cap). 0.5M sulphuric acid

DIL	**SAMP**	M480k	100mL

Sample Diluent. A weakly buffered solution formulated to expose major antigenic determinants on the adenovirus surface. Preserved with 0.099% sodium azide.

2 x resealable polythene bags
Instructions for Use

Additional Requirements:

- Disposable glass/plastic containers for stool collection
- Disposable tip micropipette to deliver volumes of 50µL
- Absorbent paper towels
- Deionised water
- Automatic plate washer or laboratory wash bottle
- Plate reader
- Range of standard clean volumetric glassware
- Range of clean, disposable plastic containers (2-30mL)
- Stoppered or screw-capped glass or plastic centrifuge tubes (approximately 10mL) capable of withstanding 1000g
- Laboratory centrifuge capable of 1000g
- Sodium hypochlorite for decontamination.
- Microgen Bioproducts Filter Pack (Cat. No M802) - optional.

WARNINGS AND PRECAUTIONS
Safety:

1. The reagents supplied in this kit are for *in vitro* diagnostic use only
2. Sodium azide, which is used as a preservative in the sample diluent can react with lead or copper plumbing to form potentially explosive metal azides. Dispose by flushing with a large volume of water to prevent azide build-up.
3. Thiomersal and sodium azide preservatives may be toxic if ingested.
4. Appropriate precautions should be taken when handling or disposing of potential pathogens. Decontamination of infectious material can be achieved with sodium hypochlorite at a final concentration of 3% for 30 minutes. Liquid waste containing acid must be neutralised before treatment.
5. The positive control has been inactivated during the manufacturing process. However, it should be handled as though potentially infectious.
6. Sulphuric acid is corrosive and should be rinsed off skin immediately after contact.

Procedural:

1. Adenoscreen® EIA should be used according to the kit instructions. Use of incubation temperatures and times other than those specified may cause erroneous results.
2. The wash buffer supplied is formulated to reduce non-specific binding which can result in elevated background absorbances. Use of any other type of wash buffer may adversely affect kit performance.
3. Allow foil/plastic microwell bags to reach room temperature before opening. This avoids formation of condensation which may contribute to deterioration of coated strips intended for future use. Do not use strips if silica gel sachet is pink as this indicates contamination with moisture.

4. Allow all reagents to reach room temperature before use.
5. Do not intermix reagents from different batches of kits.
6. Always use clean containers for wash buffer preparation.
7. Do not allow wells to dry out completely during the course of an assay.
8. Always keep the upper surface of the microtitration strips free of excess fluid droplets. Reagents and buffer overspills should be blotted dry on completion of the manipulation to avoid cross-contamination of reagents and/or samples.
9. Reagent drop deliveries should be vertically aimed at the centre of the microtitration wells.

STORAGE AND SHELF LIFE

Adenoscreen® EIA should be stored at 2-8°C when not in use. The kit should not be used after the expiry date printed on the outer carton label.
Once the original foil pack has been opened, microtitration wells should be stored in a resealable plastic bag with the sachet of desiccant. Strips can be stored at 2-8°C until the expiry date provided desiccated conditions are maintained. All liquid reagents can be used repeatedly until the dates printed on their respective labels provided they are stored at 2-8°C and that reasonable precautions are taken to avoid introducing high levels of microbial contamination.

Indications of deterioration:
Adenoscreen® EIA kits may be considered to have deteriorated if:
1. A kit consistently fails to meet the validation criteria described under **Interpretation** below.
2. Reagents spontaneously change colour, become cloudy, or develop precipitates.

SPECIMENS

Faecal specimens should be collected as soon after onset of symptoms as possible. Peak viral counts have been reported to occur 3-5 days after onset of symptoms.
The faecal sample to be tested may be collected in a clean plastic or glass container using a wooden mixing stick. Specimens may also be collected from soiled diapers. Approximately 0.1g of sample is required. Adenoscreen® EIA is not recommended for use with swab specimens as sufficient sample may not be collected. However, if there is no alternative, swabs should be eluted into 1mL of sample diluent and the test performed as normal.
The sample should not be mixed with potentially interfering substances, e.g. sera containing antibodies to adenovirus (calf or bovine sera).
Faecal specimens can be stored overnight at 2-8°C, or for longer periods at -20°C or below. Specimens containing preservatives or high detergent concentrations may yield spurious results.
To prepare the specimen for testing with Adenoscreen® EIA, add approximately 0.1g (0.1mL) of faecal material to 1.0mL Sample Diluent (M480k) and shake vigorously for 2-3 seconds. Stool suspensions containing unusually large amounts of suspended matter may require brief centrifugation (10 minutes at 1000g) to facilitate pipetting. Alternatively, suspensions may be filtered using the Microgen Bioproducts Filter Pack (M802). Faecal extracts can be stored at 2-8°C for up to 12 months. NOTE: The kit control does not require dilution.

REAGENT PREPARATION

The 20x concentrated Wash Buffer (M480e) may have formed crystals on storage at 2-8°C. These should be redissolved by standing the bottle in a 37°C water bath. Prepare working strength buffer by mixing one part concentrate with 19 parts deionised water. It is recommended that Wash Buffer sufficient only for immediate use is prepared. 150mL working strength Wash Buffer is sufficient to process one strip of 8 wells.

RINSE CYCLE

Efficient rinsing of uncomplexed components is an essential requirement of enzyme immunoassay procedures. An automatic plate washer can be used provided that it meets the following criteria:
- All wells should be completely aspirated
- All wells should be filled to the brim during the rinse cycle
- Wash buffer must be delivered at a rapid flow rate

For each rinse cycle, the machine should be set to complete 3 consecutive washes. On completion of the cycle, invert the microtitre plate and tap firmly on absorbent paper towels. **Ensure there is no residual wash buffer in the wells.** Blot dry the upper surface of the wells with a paper towel.

Alternatively, the following manual procedure may be employed:
i) Aspirate well contents using a vacuum line fitted with a trap
ii) Fill all wells to the brim with wash buffer dispensed from a squeeze-type laboratory wash bottle.
iii) Aspirate all wells
iv) Repeat steps (ii) and (iii) 3 times
v) Invert the microtitration plate and tap firmly on absorbent paper towels
vi) **Check that there is no residual fluid in the wells.**

PROCEDURE

1. Allow all reagents to reach room temperature (18-25°C) before use.
2. The positive control should be included in every assay. Sample Diluent should also be included, **in duplicate**, as a negative control.
3. Select sufficient **fresh** microwells to accommodate all test samples and controls. Fit the wells into the plastic holding frame.
4. Dispense **1 drop of Positive Control or 50µL sample diluent as negative control** into designated wells. Similarly, dispense 50µL of the extracted faecal suspensions into appropriately labelled wells.
5. Once the samples and controls have been dispensed, dispense **1 drop of conjugate** into each well, then tap the side of the plate gently to mix. Incubate at room temperature (18-25°C) for 30 minutes.
6. Aspirate and rinse the wells using the procedures recommended in "Rinse Cycle" above. Ensure complete aspiration of the wash buffer.
7. **Without delay**, dispense **2 drops of Substrate** into all wells and incubate at room temperature (18-25°C) for 20 minutes.
8. Stop the chromogenic reaction by **adding 2 drops of Stop Solution** to each well. Gently mix the well contents until the colour in the wells has changed from blue to a uniform yellow.
9. Ensure that the undersides of the wells are dry and that there are no air bubbles in the well contents. **The absorbance may be read using either dual wavelength (450nm & 610nm) or single wavelength (450nm)** using a plate reader **blanked on air**, unless the equipment manufacturer specifically recommends otherwise.

INTERPRETATION

Assay validation

An assay can be considered valid if:
i) The Positive Control Absorbance (A450) is greater than 0.6
and
ii) The mean absorbance (A450) of the two negative controls (Sample Diluent) is less than 0.15

Analysis

The cut-off value is defined as the mean absorbance (A450) of the Negative Control (Sample Diluent) plus 0.1.
Stool specimens giving absorbance values within 10% of the cut-off value are equivocal and should be repeat tested. If the result is again equivocal, a fresh sample should be obtained and tested.

A positive result is indicated when the absorbance value of the test sample is outside the equivocal range and greater than the cut-off value.
A negative result is indicated when the absorbance value of the test sample is outside the equivocal range and less than the cut-off value.

EXPECTED VALUES

A positive result indicates the presence of adenovirus antigens in the stool sample.

A negative result indicates the absence of adenovirus particles/antigens in the stool sample, or presence at levels that are lower than the limits of test sensitivity.
Adenoscreen® EIA should normally give a positive result within the first 8 days post-onset of symptoms in a true case of adenovirus enteritis. After this period, a positive result is less likely although a large proportion of cases will remain positive for considerably longer.
The rate of positivity may be expected to vary with age, weather, seasonal factors, geographical location and general health environment of the patient population under study.

LIMITATIONS OF USE

1. Adenoscreen® EIA results should be interpreted by the clinician in the context of all available clinical and laboratory information.
2. A positive Adenoscreen® EIA result does not preclude co-infection with another pathogen.
3. Faecal samples collected after the diarrhoeic phase may contain antigen concentrations below the threshold of test sensitivity.
4. A negative Adenoscreen® EIA result does not exclude the possibility of rotavirus infection. If symptoms persist or increase in intensity, another sample should be tested.

PERFORMANCE CHARACTERISTICS

Adenoscreen® EIA has been evaluated at a UK Public Health Laboratory. 150 clinical samples were tested by Adenoscreen® EIA, electron microscopy and an established commercial EIA for adenovirus. In addition, 25 viral isolates from 6 groups of common enteric viruses were tested by Adenoscreen® EIA.

Evaluation with clinical samples

Note: 1 sample was excluded as it was initially discrepant and there was insufficient sample remaining for further resolution.
Equivocal results are scored as positive.

a) Comparison of Adenoscreen® EIA with Electron Microscopy

		Adenoscreen® EIA		
		+ve	-ve	Total
Electron Microscopy	+ve	48	1*	49
	-ve	2**	98	100
	Total	50	99	149

Sensitivity: 48/49 = 98%
Specificity: 98/100 = 98%
Diagnostic Efficiency: 146/149 = 98%

*1 sample positive by E.M. and commercial EIA

** 1 sample negative by E.M. and commercial EIA
1 sample negative by E.M., positive by commercial EIA (sample recovered from soiled diaper)

b) Comparison of Adenoscreen® EIA with an established commercial EIA

		Adenoscreen® EIA		
		+ve	-ve	Total
Commercial EIA	+ve	48	1*	49
	-ve	2+	98	100
	Total	50	99	149

Sensitivity: 48/49 = 98%
Specificity: 98/100 = 98%
Diagnostic Efficiency: 146/149 = 98%

*1 sample positive by E.M. and commercial EIA

+ 1 sample negative by E.M. and commercial EIA
1 sample positive by E.M., negative by commercial EIA and equivocal by Adenoscreen® EIA. (Sample from a child who had received a bone marrow transplant)

Evaluation with viral isolates

A range of common enteric viruses (other than adenovirus) was tested with Adenoscreen® EIA to confirm the specificity of the product.

No cross reactivity was seen with any of the viral isolates in the test panel listed below:

Rotavirus (7 isolates)
Coronavirus (5 isolates)
Astrovirus (6 isolates)
Small round structured viruses (SRSV) genotypes 1 and 2 (5 isolates)
Coxsackie B4 (1 isolate)
Poliovirus Type 1 (1 isolate)

Evaluation with bacterial isolates

A range of bacterial isolates was tested with Adenoscreen® EIA to check for potential cross-reactivity with these organisms.

No cross-reactivity was seen with:
Acinetobacter baumannii
Aeromonas hydrophila
Bacillus subtilis
Campylobacter
Candida albicans
Citrobacter
Enterobacter aerogenes
Escherichia coli
Haemophilus influenzae
Klebsiella aerogenes
Listeria monocytogenes 2b
Proteus mirabilis
Proteus vulgaris
Pseudomonas aeruginosa
Salmonella typhimurium
Shigella sonnei
Streptococcus Group B
Yersinia enterocolitica (Myf+ and Myf-)

Staphylococcus aureus and *Streptococcus Group A* did not cross-react at concentrations less than 10^9 cfu/mL. Above this concentration, however, some cross-reactivity was evident.

REPRODUCIBILITY

Intra-assay reproducibility (repeatability) - This was evaluated by testing multiple replicates of kit positive control and two reference samples (high and low positive titre) on 5 separate occasions. The intra-assay coefficient of variation for the kit positive control ranged from 5.6-10.0% and, for the high and low titre reference samples, 4.9-7.8% and 3.7-6.9% respectively.

Inter-assay reproducibility - This was evaluated by testing multiple replicates of kit positive control and two reference samples (high and low positive titre) on 5 separate occasions. The overall inter-assay coefficient of variation for the kit positive control was 12.7% and, for the high and low titre reference samples, 12.9% and 18.4% respectively.

KEY diagnostics
Tel : 02 8212 4074
Fax:02 9423 6992
PO Box 1038
Gymea NSW 2227
info@keydiagnostics.com.au
www.keydiagnostics.com.au