DRG® Rotavirus Ag ELISA (EIA-4455)

REVISED 28 OCT. 2013 RM (VERS. 9.1) **USA: RUO**

This kit is intended for Research Use Only.

Not intended for use in diagnostic procedures.

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

The Rotavirus Ag ELISA is a device for direct detection of Rotavirus in faecal samples.

2 PRINCIPLE OF THE TEST

The Rotavirus Ag ELISA is a one-step enzyme immunoassay on the basis of polyclonal antibodies to the group specific VP-6 antigen, the major protein of group A Rotaviruses.
Diluted stool specimens and horseradish peroxidase (HRP) labelled polyclonal anti-Rotavirus-antibodies are dispensed simultaneously into the wells of a microtitration plate coated with polyclonal anti-Rotavirus antibodies.
After an incubation time of 60 min at room temperature unbound components are removed by a washing step.
HRP converts the subsequently added colorless substrate solution of 3,3',5,5'-Tetramethylbenzidine (TMB) within a 10 min reaction time into a blue product. The enzyme reaction is terminated by sulphuric acid dispensed into the wells turning the solution from blue to yellow.
The optical density (OD) of the solution read at 450/620 nm is directly proportional to the specifically bound amount of Rotavirus.

3 TEST COMPONENTS FOR 96 WELLS

1	WELLS	**Microtitration plate** coated with polyclonal anti-Rotavirus-antibodies (sheep)	12 single breakable 8-well strips colour coding dark blue vacuum-sealed with desiccant
2	WASHBUF CONC 10x	**Wash buffer** 10-fold	100 mL concentrate for 1000 mL solution white cap
3	DIL	**Sample diluent**	100 mL · ready to use coloured yellow black cap
4	CONTROL +	**High control** *Rotavirus* reactive sample	1.5 mL · ready to use coloured blue red cap
5	CONTROL –	**Low control** *Rotavirus* low sample	1.5 mL · ready to use coloured blue green cap
6	CONJ HRP	**HRP-conjugate** HRP-labelled, polyclonal anti-Rotavirus-antibodies (rabbit)	12 mL · ready to use coloured green brown cap
7	SUBSTR TMB	**Substrate** 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide	15 mL · ready to use blue cap
8	STOP	**Stop solution** 0.25 M sulphuric acid	15 mL · ready to use yellow cap

4 PREPARATION AND STORAGE OF SAMPLES

4.1 Collection and storage

Stool samples should be stored at 2 °C - 8 °C immediately after collection and processed within 72 hours. Longer storage is possible at -20°C. Repeated freezing and thawing of samples should be avoided.
Stool samples already diluted with the sample diluent (DIL) can be stored for up to 72 h at 2 °C - 8 °C before testing in the ELISA.

4.2 Preparation

Quickly thaw frozen samples; Warm samples to room temperature and mix well.
The Rotavirus ELISA (EIA-4455) can be performed with 1:6 or 1:11 diluted specimens. In case of additional testing of the same sample in the Clostridium difficile Toxin A+B ELISA (EIA-4448) the 1:6 dilution is recommended.

Preparation of a 1:11 sample dilution:
Pipette **1000 µL** of sample diluent into a clean tube. Using a disposable stirring rod transfer about **100 mg** (diameter about 2-3 mm) of faeces if solid or pipette **100 µL** if liquid into the tube and suspend thoroughly.
If necessary, sediment floating particles by a centrifugation step with a micro centrifuge for one min at maximum speed.

Preparation of a 1:6 sample dilution:
Pipette **1000 µL** of sample diluent into a clean tube. Using a disposable stirring rod transfer about **200 mg** (diameter about 4-6 mm) of faeces if solid or pipette **200 µL** if liquid into the tube and suspend thoroughly.
If necessary, sediment floating particles by a centrifugation step with a micro centrifuge for one min at maximum speed.

5 MATERIALS REQUIRED BUT NOT PROVIDED

- Micropipettes
- Multi-channel pipette or multi-pipette
- Reagent container for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- Microplate reader with optical filters of 450 nm for measurement and ≥ 620 nm for reference
- Distilled or deionized water
- Glassware
- Tubes (2 mL) for sample preparation

6 PREPARATION AND STORAGE OF REAGENTS

6.1 Kit size and expiry

One kit is designed for 96 determinations.
The expiry date of each component is reported on its respective label, that of the complete kit on the outer box label.
Upon receipt, all test components have to be kept at 2 °C - 8 °C, preferably in the original kit box.
After opening all kit components are stable for at least 2 months, provided proper storage.
The ready to use wash buffer solution is stable for at least 1 month when stored at 2 °C - 8 °C.

6.2 Reagent preparation

Allow all components to reach room temperature prior to use in the assay.
The microtitration plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed plate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the 10-fold concentrated wash buffer **1 + 9** with distilled or deionized water.

For Example:
10 mL wash buffer concentrate + 90 mL distilled or deionized water.

7 ASSAY PROCEDURE

- Dilute samples with sample diluent (3) 1:11 or 1:6,
  e.g.
  100 mg or 100 µL stool + 1.0 mL (**1:11**) sample diluent (3)
  or
  200 mg or 200 µL stool + 1.0 mL (**1:6**) sample diluent (3)
- Avoid any time shift during dispensing of reagents and samples.
- Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle and that the remaining fluid is completely drained in every single wash cycle!
- Avoid light exposure of the TMB substrate solution!

7.1 Working steps

1. Warm all reagents to room temperature before use. Mix gently without causing foam.
2. Dispense **2 drops (or 75 µL)** CONJ HRP HRP-conjugate (6) per well and
3. **Pipette**
   75 µL CONTROL + High control (4)
   75 µL CONTROL - Low control (5)
   50 µL diluted sample, mix gently
4. Cover plate and incubate for **60 min** at room temperature.
5. Decant, then wash each well **5x** with **300 µL** wash solution (diluted from (2)) and tap dry onto absorbent paper.
6. Dispense **2 drops (or 75 µL)** SUBSTR TMB substrate (7) per well.
7. Incubate for **10 min** at room temperature protected from light.
8. Dispense **2 drops (or 75 µL)** STOP stop solution (8) per well, mix gently.
9. Read OD at **450 nm** /≥ 620 nm with a microplate reader within 30 min after reaction stop.

8 RESULT INTERPRETATION

Qualitative evaluation

Cut-off determination: **OD low control + 0.20**

Samples with OD values equal with or higher than the cut-off are considered **high**,

samples with OD values below the cut-off are considered **low** for Rotavirus antigen .

8.1 Limitations of the procedure

There is no correlation between measured absorbance and seriousness of the infection. It is also not allowed to correlate absorbances of the samples with that of the high control.
Cross contamination of reagents and samples can produce false high results. Incorrect dilutions, not sufficiently homogenized samples or solid particles after centrifugation of the suspension can cause false negative as well as false high results.
A low test result not necessarily excludes a Rotavirus infection. Inhomogeneous virus distribution in the sample can cause false low results. The investigation of samples that were taken beyond the acute phase of the disease can cause false low results, because the number of virus particles has decreased under the detection limit of the test. It is therefore recommended to take samples within the acute phase of the disease where a maximum number of excreted virus particles are to be expected. Faecal samples from vaccinated children may contain vaccine virus causing high ELISA results.

8.2 Automatic Processing

Performing the Rotavirus Ag ELISA on fully automated microplate processors (e.g. DS2, DSX) may cause elevated absorbances in comparison to the manual procedure due to individual differences concerning wash procedures and general technical specifications of the equipment. In these cases a maximum value of 0.3 absorbance units is permissible for the low control.
It is recommended to use a wash procedure including 10 seconds soak time per strip and wash step followed by a wash step with distilled or deionized water with 10 seconds of soak time after the final wash step of each wash cycle. If necessary the number of washing steps can be enhanced from 5x to 7x-8x.

Correlation: Manual – automatic processing
A panel of 133 stool specimens was investigated in parallel by manual and automatic processing method (DS2, Dynex Technologies) resp. The correlation was calculated with r = 0.96.

9 COMMON ADVICES AND PRECAUTIONS

Follow the working instructions carefully. The kit should be performed by trained technical staff only.
The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
Do not use or mix reagents from different lots except for sample diluent, wash buffer, TMB/substrate solution and stop solution.
Do not use reagents from other manufacturers.
Avoid time shift during dispensing of reagents.

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All reagents should be kept at 2 °C - 8 °C before use.
Some of the reagents (2,3,4,5,6,7) contain small amounts of Thimerosal (< 0.01 % w/v) and Kathon (1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucous membranes.
Handle all components and all samples as if potentially hazardous.
Since the kit contains potentially hazardous materials, the following precautions should generally be observed:

- Do not smoke, eat or drink while handling kit material,
- Always use protective gloves,
- Never pipette material by mouth,
- Note safety precautions of the single test components.

References

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Rev. 10/10/13cc