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# DRG<sup>®</sup> HAV Ab (EIA-4233)

## Revised 16 Sept. 2010 rm (Vers. 3.1)

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

#### **INTENDED USE**

Competitive Enzyme ImmunoAssay (ELISA) for the determination of antibodies to Hepatitis A Virus in human plasma and sera.

For Research Use Only.

#### **PRINCIPLE OF THE TEST**

The assay is based on the principle of competition where the antibodies in the sample compete with an anti-HAV specific antibody, labeled with HRP, for a fixed amount of antigen on the solid phase.

A purified and inactivated HAV is coated to the microwells. The donor's serum/plasma is added to the microwell and antibodies to HAV are captured by the solid phase.

After washing, the enzyme conjugate is added and binds to the free HAV antigen, if still present.

The plate is washed to remove unbound conjugate and then the chromogen/substrate is added.

In the presence of peroxidase the colorless substrate is hydrolysed to a coloured end-product, whose optical density may be detected and is inversely proportional to the amount of antibodies to HAV present in the sample.

An additive is added to the sample directly into the well to block interferences able to mask the presence of antibodies, mostly appearing in the follow up of vaccination.

#### **COMPONENTS**

Each kit contains sufficient reagents to perform 96 tests.

#### **1. Microplate** MICROPLATE

8x12 microwell strips coated with purified and inactivated HAV, sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening. Reseal unused strips in the bag with desiccant and store at  $2..8^{\circ}$ C°.

#### 2. Negative Control: CONTROL –

1 x 2. 0ml/vial. Ready to use.

Contains bovine serum proteins, 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.1% Kathon GC as preservatives. The negative control is color coded pale yellow.

#### **3. Positive Control**: CONTROL +

1 x 2.0 ml/vial. Ready to use.

Contains bovine serum proteins, anti HAV antibodies at a concentration higher than 100 WHO mIU/ml, 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.1% Kathon GC as preservatives. The positive control is colour coded green.





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4. Calibrator: CAL ....

n° 1 vial. Lyophilized.

To be dissolved with EIA grade water as reported in the label.

Contains bovine serum proteins, anti HAV antibodies at a concentration of about 10 WHO mIU/ml, 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.1% Kathon GC as preservatives. Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

# **5. Wash buffer concentrate**: WASHBUF 20X

1 x 60 ml/bottle. 20x concentrated solution. to be diluted up to 1200ml with distilled water before use. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.05% Kathon GC.

# 6. Enzyme conjugate: CONJ

1 x 16 ml/vial. Ready-to-use solution.

Contains Horseradish peroxidase conjugated antibody, specific to HAV, in presence of 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives. The reagent is colored with a red dye.

### 7. Chromogen/Substrate: SUBS TMB

1 x 16ml/vial.

Contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide of H<sub>2</sub>O<sub>2</sub>.

# Note: To be stored protected from light as sensitive to strong illumination.

# **8. Specimen Diluent:** DILSPE

1 x 8ml.

Buffered solution suggested to be used in the follow up of vaccination. It contains 0.09% sodium azide and 0.1% Kathon GC as preservatives. The reagent is color coded dark green.

# **9.** Sulphuric Acid: $H_2SO_4 O.3 M$

1 x 15 ml/vial. Contains 0.3 M H<sub>2</sub>SO<sub>4</sub> solution. Attention: Irritant (Xi R36/38; S2/26/30)

# 10. Plate sealing foils

### 11. Package insert

Upon request:

### **Calibration Curve:**

5 x 2.0 ml/vial. Ready to use and colour coded standard curve ranging: 0-5-10-50-100 WHO mIU/ml. (CAL1=0mIU/ml, CAL2=5mIU/ML, CAL3=10mIU/ml, CAL4=50mIU/ml, CAL5=100mIU/ml). Contains serum proteins, 0.3 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.





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Standards are blue colored.

### MATERIALS REQUIRED BUT NOT PROVIDED

- 1. Calibrated Micropipettes (150  $\mu$ L, 100  $\mu$ L and 50  $\mu$ L) and disposable plastic tips.
- 2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
- 3. Timer with 60 minute range or higher.
- 4. Absorbent paper tissues.
- 5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C (+/-0.1°C tolerance).
- 6. Calibrated ELISA microwell reader with 450nm (reading) and if possible with 620-630nm (blanking) filters.
- 7. Calibrated ELISA microplate washer.
- 8. Vortex or similar mixing tools.

#### SPECIMEN: PREPARATION AND RECOMMANDATIONS

- 1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
- 2. Avoid any addition of preservatives; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.
- 3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and reading is strongly recommended.
- 4. Haemolysed and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
- 5. Sera and plasma can be stored at +2°C 8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at -20°C for several months. Any frozen samples should not be freeze/thawed more than once as this may generate particles that could affect the test result.
- 6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8  $\mu$  filters to clean up the sample for testing.

#### PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on opened kit has pointed out no relevant loss of performances up to 3 months from first opening.

#### 1. Antigen coated microwells:





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Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in manufacturing. In this case, call DRG's customer service.

Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at  $+2^{\circ}-8^{\circ}C$ .

When opened the first time, unused strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

#### 2. Negative Control:

Ready to use. Mix well on vortex before use.

#### 3. Positive Control:

Ready to use. Mix well on vortex before use.

#### 4. Calibrator:

Add the volume of ELISA grade water, reported on the label, to the lyophilized powder; let fully dissolve and then gently mix on vortex. The dissolved calibrator is not stable; store it frozen in aliquots at  $-20^{\circ}$ C.

#### 5. Wash buffer concentrate:

The whole content of the 20x concentrated solution has to be diluted with bi-distilled water up to 1200 ml and mixed gently end-over-end before use.

Once diluted, the wash solution is stable for 1 week at 2-8° C.

During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.

#### 6. Enzyme conjugate:

Ready to use. Mix well on vortex before use.

Avoid contamination of the liquid with oxidizing chemicals, dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

#### 7. Chromogen/Substrate:

Ready to use. Mix well on vortex before use.

Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.

If this component has to be transferred use only plastic, and if possible, sterile disposable container

#### 8. Specimen Diluent:

Ready to use. Mix well on vortex before use.

#### 9. Sulphuric Acid:

Ready to use. Mix well on vortex before use. Attention: Irritant (Xi R36/38; S2/26/30) Legenda: R 36/38 = Irritating to eyes and skin. S 2/26/30 = In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

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## INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

- Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained. Decontamination of spills or residues of kit components should also be carried out regularly. They should also be regularly maintained in order to show a precision of 1% and a trueness of +2%.
- 2. The ELISA incubator has to be set at +37°C (tolerance of +/-0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
- 3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls and reference panels, before using the kit for routine laboratory tests. 4-5 washing cycles (aspiration + dispensation of 300ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the sections "Validation of Test" and "Assay Performances". Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
- 4. Incubation times have a tolerance of  $\pm 5\%$ .
- 5. The ELISA reader has to be equipped with a reading filter of 450nm and ideally with a second filter (620-630nm) for blanking purposes Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
- 6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Validation of Test" and "Assay Performances". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.

#### PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.





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- 2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate (TMB+H<sub>2</sub>O<sub>2</sub>) is colorless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
- 3. Dilute all the content of the 20x concentrated Wash Solution as described above.
- 4. Dissolve the Calibrator as described above and gently mix.
- 5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
- 6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturer's instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.
- 7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
- 8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
- 9. Check that the micropipettes are set to the required volume.
- 10. Check that all the other equipment is available and ready to use.
- 11. In case of problems, do not proceed further with the test and advise the supervisor.

### ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

1. Place the required number of strips in the microplate holder. Leave A1 well empty for the operation of blanking.

Store the other strips into the bag in presence of the desiccant at  $+2^{\circ}$ C -  $8^{\circ}$ C, sealed.

 Dispense 50 μL Specimen Diluent in all the wells identified for samples and controls/calibrator, except for A1.

Then pipette 100  $\mu$ l of Negative Control in triplicate, 100  $\mu$ l of Calibrator in duplicate, 100  $\mu$ l Positive Control in single and then 100  $\mu$ L of samples.

Check that controls/calibrator and samples have been correctly added.

Incubate the microplate at +37°C for 60 min.

- 3. Wash the microplate as reported in section 9.3.
- 4. In all the wells except A1, pipette 100 μl Enzyme Conjugate. Check that the reagent has been correctly added. Incubate the microplate at +37°C for 60 minutes. *Important note:* Be careful not to touch the inner surface of the well with the pipette tip when dispensing the Enzyme Conjugate. Contamination might occur.
- 5. Wash the microplate as described.







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- 6. Pipette 100 μl TMB/H<sub>2</sub>O<sub>2</sub> mixture in each well, the blank wells included. Check that the reagent has been correctly added. Then incubate the microplate at room temperature for 20 minutes. *Important note:* Do not expose to strong direct light as a high background might be generated.
- Pipette 100 µl Sulphuric Acid into each well to stop the enzymatic reaction using the same pipetting sequence as in step 6. Then measure the color intensity with a microplate reader at 450nm (reading) and possibly at 620-630nm (blanking), blanking the instrument on A1 well.

#### Important notes:

- 1. If the second filter is not available, ensure that no finger prints are present on the bottom of the microwell before reading at 450nm. Finger prints could generate false positive results on reading.
- 2. Reading has should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.

50 µL
100 µL
100 µL
60 min
+37°C
4-5 cycles
100 µL
60 min
+37°C
4-5 cycles
100 µL
20 min
r.t.
100 µL
450nm & 620nm

#### ASSAY SCHEME





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An example of dispensation scheme is reported in the table below:

	Microplate													
	1	2	3	4	5	6	7	8	9	10	11	12		
Α	BLK	S2												
В	NC	S3												
С	NC	S4												
D	NC	S5												
Е	CAL	S6												
F	CAL	S7												
G	PC	S8												
Н	S1	S9												

Legenda: BLK = Blank NC = Negative Control CAL = Calibrator PC = Positive Control S = Sample

### **CALCULATION OF THE CUT-OFF**

The test results are calculated by means of a cut-off value determined with the following formula: Cut-Off = (NC+PC) / 3

The value found for the test is used for the interpretation of results as described in the next paragraph.

*Important note*: When the calculation of results is performed by the operating system of an ELISA automated work station, make sure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.

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