DRG® Tuberculosis IgA // IgM // IgG (EIA-4250 // EIA-4251 // EIA-4252)

CE

Revised 30 Sept. 2010 rm (Vers. 3.1) **RUO in the USA**

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

Intended Use

DRG Tuberculosis ELISA has been designed for the detection of IgG / IgM / IgA antibodies against Mycobacterium tuberculosis in serum and plasma. Further applications in other body fluids are possible and can be provided on request.

This assay is intended for Research use only.

Principle of the Test

The principle of the test reaction can be described in four stages.

1. Serum incubation

Specific antibodies bind to the antigens on the solid phase to form a stable immune complex.
After a 60 minutes incubation at room temperature the wells are washed with prediluted wash buffer to remove all non-reactive serum components.

2. Conjugate incubation

The anti-human-IgG /-IgM /-IgA horseradish peroxidase conjugate is added to all wells. The conjugate binds to IgG / IgM / IgA antibodies on the solid phase antigen to form a stable sandwich. After a 30 minutes incubation at room temperature the excess conjugate is removed by washing all wells with washing buffer.

3. Substrate reaction and stopping

The TMB substrate is dispensed into each well and the peroxidase enzyme/substrate reaction forms a stable blue chromogen. The reaction and subsequently the colour development is stopped after 20 minutes incubation at room temperature by adding 0.5 M H_2SO_4 to the wells. The change in pH also causes the chromogen to change colour from blue to yellow.

4. Reading and interpretation

The intensity of the colour is read in a microtiter plate reader at 450 nm (recommended reference wavelength for bichromatic measurement: 600–690 nm). The intensity of the colour (OD) is directly proportional to the concentration of the specific antibody in the donor sample.

Kit Contents

The kits contains sufficient reagents for 12 x 8 = 96 determinations.

The strips and solutions have to be stored at 2–8 °C. The expiry date is mentioned on the labels.

12	**Microtiter strips**	single strips each with 8 break-apart wells coated with the antigen of Mycobacterium tuberculosis
1 x	**Frame holder**	
4 x 2 mL	**Standards 1–4**	human serum containing antibodies against Mycobacterium tuberculosis (concentrations listed below) diluted in PBS and stabilised with 0.01 % methyliso-thiazolone and 0.01 % bromonitrodioxane as preservatives, ready to use

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		IgG	IgM	IgA
Std.. 1 (negative)	Concentration (U/mL)	1	1	1
Std.2 (Cut-off)		10	10	10
Std. 3 (weak positive)		40	30	30
Std. 4 (positive)		150	100	100

1 x 60 mL	**Serum Diluent**	PBS/BSA buffer solution, contains < 0.1 % sodium azide as preservative, ready to use
1 x 12 mL	**Enzyme Conjugate**	HRP-labelled goat anti-human-IgG /-IgM /-IgA, ready to use
1 x 12 mL	**TMB Substrate Solution**	3,3',5,5' Tetramethylbenzidine, ready to use
1 x 12 mL	**Stop Solution**	0.5 M sulfuric acid, ready to use
1 x 60 mL	**Wash Buffer** 10 x concentrated	PBS/Tween buffer solution 10 x concentrated to be diluted 1:10 prior to use; the concentrate should be warmed up to 37 °C for 15 minutes to avoid any crystals
2 x	**Plate sealers**	to cover microtiter strips during incubation
1 x	**Plastic bag**	re-sealable for dry storage of non-used strips

Materials Required but not Provided

- 5 µL-, 100 µL- and 500 µL micro- and multichannel pipets
- Microtiter plate reader with a 450 nm filter (reference filter 600–690 nm)
- Microtiter Plate Washer (in case of manual washing: wash bottle)
- Reagent tubes for the serum dilution
- Measuring cylinder
- Distilled water or water of higher quality

Warning and Precautions

1. For research use only! Do not ingest or swallow! Laboratory safety precautions should be followed. Do not eat, drink or smoke in the laboratory.
2. All sera and plasma or buffers based upon have been tested to HBsAg, HIV and HCV respectively with generally accepted methods and were found negative. Nevertheless, precautions like the use of latex gloves have to be taken.
3. 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.
4. All reagents have to be brought to room temperature (18 to 24 °C) before performing the test.

5. Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
6. It is important to pipet with constant intervals so that all the wells of the microtiter plate have the same conditions.
7. 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.
8. In order to avoid a carry-over or a cross-contamination separate disposable pipet tips have to be used.
9. No reagents from different kit lots should be used and they should not be mixed with one another.
10. All reagents have to be used within shelf life.
11. In accordance with a Good Laboratory Practice (GLP) or following ISO 9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers e.g. to microliter pipets and washing or reading (ELISA Reader) instrumentation.
12. The contact of certain reagents especially 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.

Storage and Stability

Store all reagents at 2–8 °C.
The expiry date of each reagent is printed on the individual labels. Do not use any reagents after the expiry date has been exceeded.
The diluted washing buffer is stable for up to 4 weeks when stored at 2–8 °C.
The opened kit should be used within three months.

Specimen Collection and Handling

Both 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 at 2–8 °C for up to 3 days. They should be kept at -20°C for a longer storage. The samples should not be frozen and thawed repeatedly.
Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.
Donor sera must be **prediluted 1:101** in Serum Diluent (e.g. 5 µL serum + 500 µL Serum Diluent) prior to testing.
Samples containing concentrations higher than the highest standard have to be diluted further with Serum Diluent.
In case of interference with rheumatic factors, serum preabsorption with RF absorbent REF 651003) is recommended. Do **not** absorb the standards.

Assay Procedure

Preparation of Reagents: Allow all kit components and specimens to reach room temperature (RT, 18–24 °C) prior to use and mix well.

Wash Buffer:
Dissolve any crystals which may be in the bottle by warming to 37 °C and then mix well.

Dilute the concentrated washing buffer 1:10 with distilled water (e.g. 60 mL buffer concentrate + 540 mL distilled water). Mix thoroughly.

- Strictly follow the instructions for reliable test performance. Any changes or modifications are within the responsibility of the user.
- All reagents and samples must be brought to room temperature before use, but should not be left at this temperature for longer than necessary.
- A standard curve should be established with each assay.
- Put the unused microtiter strips back in the plastic bag and store them dry at 2–8 °C.

Assay Steps

Prepare a sufficient amount of microtiter wells for standards, controls and samples.

Note:
Other incubation conditions might be possible. In case of modifications of the recommended test procedure (e.g. incubation temperature 37 °C instead of RT) the user has to validate assay performance.

1. Pipette 100 µL each of the diluted (1:101) samples and the ready to use standards into the appropriate wells.
2. Cover plate with the enclosed plate sealing foil and incubate at room temperature for 60 minutes.
3. Discard the contents of the microwells and wash 3 times with 300 µL of diluted washing buffer. Afterwards remove residues of the washing solution by gentle tapping of the microtiter plate on a paper towel.
4. Pipette 100 µL of enzyme conjugate solution into each well.
5. Cover plate with plate sealing foil and incubate for 30 minutes at room temperature.
6. Discard the contents of the microwells and wash 3 times with 300 µL of diluted washing buffer. Afterwards remove residues of the washing solution by gentle tapping of the microtiter plate on a paper towel.
7. Dispense 100 µL of TMB substrate into each well.
8. Cover plate with the plate sealing foil and incubate for 20 minutes in the dark (e.g. drawer) at room temperature.
9. Add 100 µL of stopping solution to each well.
10. After thorough mixing and wiping the bottom of the plate, read the optical density at 450 nm and calculate the results. Blank against air. A bichromatic measurement using a reference wavelength of 600–690 nm is recommended.
    The developed colour is stable for at least 60 minutes. Read optical densities during this time.

Results and Interpretation

Example

	OD 450 nm	corrected OD	Mean OD Value
Blank	0.020		
Standard 1 (negative)	0.024/0.026	0.004/0.006	0.005
Standard 2 (cut-off)	0.520/0.580	0.500/0.560	0.530
Standard 3 (weak positive)	1.100/1.132	1.080/1.112	1.096
Standard 4 (positive)	1.500/1.590	1.480/1.570	1.525

The table above should be considered as an example which was achieved under arbitrary temperature and environmental conditions. These data do NOT describe **reference values** which have to be found in other laboratories in the same way!

Qualitative Calculation

The calculated OD values for donor sera as mentioned above are compared with the value for the cut-off standard. If the value of the sample is higher, then it should be read as *positive*.
A value below the cut-off standard should be read as *negative*.
It seems reasonable to define a range of ± 20 % around the value of the cut-off as a *grey zone*. It is recommended to repeat results laying within the grey zone using the same serum or a new sample of the same donor, taken after 2–4 weeks. Both samples should be measured in parallel in the same run.
The positive standard must show an absorption value at least double the value received by the cut-off standard.

Quantitative Calculation

The ready to use standards of the Mycobacterium tuberculosis antibody kit are defined and values expressed are in arbitrary units (U/mL). This gives access to an exact and reproducible quantification and in consequence donor antibody titer monitoring is possible. Concentration values for standards are printed on the labels of the vials.
A standard curve is plotted by entering the mean absorbance value of the standards on the Y-axis and the corresponding concentration on the X-axis using graph paper. The concentration of the donor samples can then be read directly from the graph.
The calculation of the result can be performed using a computer and a suitable software program.

References

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