MEDICAL BIOLOGICAL UNION

For *in vitro* diagnostic procedure use only

Measles-IgG-DS

For the detection and quantitative determination of IgG antibodies to measles virus in human blood serum or plasma

Cat. # D-402

96 tests

Store at 2–8°C.
All kit components are stable until the expiration date printed on the label.

Instruction Manual

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1 Intended use

Measles-IgG-DS is an enzyme-linked immunosorbent assay (ELISA) for the detection and quantitative determination of IgG antibodies to measles virus in human blood serum or plasma and is intended for aid in the diagnosis of patients suspected of having measles or in the estimation of the vaccination anti-measles success.

2 Introduction

Measles is the most contagious disease known to man. The measles virus is an RNA paramyxovirus of the Morbillivirus genus. The virus is spread by respiratory droplets (11.1).

Measles is sometimes complicated by ear infections, pneumonia, or encephalitis (inflammation of the brain), which can lead to convulsions, deafness, or mental retardation (11.2). A woman who gets measles during pregnancy has an increased risk of having a premature baby or a baby that dies before birth (11.3)

The first symptoms of measles start about 10 days after exposure and the illness lasts from 1 to 2 weeks. The illness starts with a runny nose, watery eyes, cough, and high fever. In the first few days, tiny, white spots, appear in the mouth. After 2 to 4 days, a raised, red rash starts on the face and spreads down the body and out to the arms and legs. The rash usually lasts 4 to 7 days and appears about 14 days after exposure. People with measles are contagious for 1 week before and at least 4 days after the rash begins (11.4).

Because of symptoms of measles is the similar with other infections, it is necessary to make the discriminating diagnosis before treatment. Monitoring of immunity to measles relies solely on detection specific antibodies by immunosorbent assays ELISA (11.5,11.6,11.7) This method allows to determinate recent infection and immunity to measles (11.8).

3 Principle of the procedure

The wells of the 96-well plate are coated with inactivated cleared antigen and recombinant proteins of measles virus. The mouse anti-human IgG monoclonal antibodies conjugated with horse-radish peroxidase serve as the conjugate.

IgG antibodies contained in the test sample which are specific for the measles virus bind to the antigen in the wells of the test plate. The conjugate binds to these specific antibodies. The enzyme component of the conjugate catalyses the reductive cleavage of hydrogen peroxide and a chromogen (3,3',5,5'Tetramethylbenzidine, TMB chromogen) added to the reaction is oxidized by the peroxidase enzyme to yield a blue-colored reaction complex. This reaction is terminated by the addition of stop solution (colour changes to yellow).

The optical density of the solutions in the wells at 450 nm is formed proportional to the activity of the virus-specific IgG antibodies contained in the sample.

4 Reagents and materials

4.1 Components in each 96-test kit of Measles-IgG-DS

1 block	**ELISA 96-well stripped plate** 12 strips in *yellow* frame per plate, each containing 8 wells coated with inactivated cleared antigen and recombinant proteins of measles virus. Prepare for use as described.	
1 x 26 ml	**Wash buffer concentrate (PBS-T)** A phosphate-saline buffered solution supplemented with Tween	Transparent opalescent colorless liquid
1 x 12 ml	**Serum dilution buffer (SB)** Diluting buffered solution for Sera	Transparent colorless opalescent liquid
1 x 12 ml	**Serum preliminary dilution buffer (SBP)** Buffered solution for preliminary dilution of Sera	Transparent yellow - orange liquid
1 x 12 ml	**Conjugate dilution buffer (CgB)** Diluting buffered solution for conjugate	Transparent opalescent green liquid
1 x 13 ml	**Substrate buffer (BS TMB)** Citrate-phosphate solution for substratum with hydrogen peroxide	Transparent colorless liquid
1 x 0.5 ml	**Conjugate (Cg)** The mouse anti-human IgG monoclonal antibodies which has been conjugated with horse-radish peroxidase	Transparent viscous yellow liquid
1 x 2 ml	**Positive control (C+)** Inactivated Serum of human blood containing human IgG specific for measles virus	Transparent red liquid
1 x 3 ml	**Negative control (C-)** Inactivated human blood serum containing no anti- measles virus IgG	Yellow transparent liquid
1 vial with tablet	**Calibrator** Lyophilized inactivated Serum of human blood, containing human IgG specific for measles virus	Yellow tablet
1 x 0.5 ml	**TMB chromogen (TMB)** 3,3',5,5'Tetramethylbenzidine dissolved in DMSO	Colorless or light yellow liquid
1 x 6 ml	**Stop solution** Sulfuric acid 0.9 mol/l.	Colorless transparent liquid
1 block	**The plate for preliminary serum dilution** 12 strips in *white* frame per plate	

The kit is intended for assaying 96 samples, including control samples. For research of a small number of samples: 12 ELISA runs on 8 analyses each are possible, including control samples.

4.2 Additional materials and instruments required

- Freshly distilled or de-ionised water.
- Disposable gloves.
- Timer.
- 76 % ethanol, hydrogen peroxide (6%) or other disinfectants.
- Appropriate biohazard waste container for potentially contaminated materials.
- Absorbent tissue.
- Dispensing system and/or disposable tip pipettes (single or multichannel) with an accuracy of ±5%, and tips.
- Thermostat, 37±1 °C.
- Micro well reader, single wavelength 450±5 nm as reference with a linear absorbance range of 0 to 2.000 or higher.

- Micro well aspiration/wash system capable of containing aspirated waste in a closed container and capable of filling and aspirating the wells completely without overflowing (recommended).
- Film for strips sealing.
- Clean bottles in capacity of 10-20 ml.

5 Safety precautions and warnings

In spite of the fact that components of a kit Measles-IgG-DS prepared of a donor's blood have passed the corresponding control and have shown absence of antibodies to the HIV-1 and the HIV-2, to viruses of hepatitises B and C, *Treponema pallidum*, and also HBs-antigen, and have been inactivated, they and also researched samples of patients should be treated as potentially infected.

Therefore during performance of the analysis it is necessary to keep all precautions accepted at work with potentially infectious material:

- Use rubber gloves for all the manipulations;
- Never pipette any solutions by mouth;
- Treat all the waste material with 76% (v/v) ethanol or 6% (w/w) hydrogen peroxide followed by exposure at 20–25°C for at least 2 hours;
- Immediately clean up any spillage containing potentially infectious agents with disinfectants. Dispose of the cleaning material by an accepted method;
- Dispose of all specimens and materials used to perform the test as they contain infectious agents. Use one of the following methods:
    - Autoclave for 60 minutes at 121 °C.
    - Incinerate disposable materials.
    - Mix liquid waste with 6% hydrogen peroxide. Allow it to stand 30 minutes before disposal.

Note! *Never use components from kits of different lots or mix such components while preparing solutions.*

Oxidizing agents, metal ions, or detergents on glass- and plastic ware may degrade TMB. To avoid false results, pay attention to careful washing of all the glass and plastic ware with sulfuric acid (1 mol/l) or hydrochloric acid (1 mol/l) followed by thorough washing with distilled water.

It is necessary to use clean measured utensils and automatic pipettes with a margin error gaugings of volumes no more than 5% for preparation of reagents and ELISA performing.

Note! *Do not confuse the strips of the Plate for preliminary serum dilution and plate coated with antigen.*

6 Specimen collection and preparation

No special preparation or fasting of the patient is necessary.

Serum or plasma may be used. No adverse affects were observed using citrate, heparin or EDTA as anticoagulants.

Blood should be collected by normal venipuncture technique and handled with proper precautions in accordance with standard laboratory procedures.

Specimens containing sodium azide or particulate matter may give erroneous results.

Specimens with moderately elevated levels of bilirubin, haemoglobin, lipids or proteins do not affect the test result.

Do not use the sera samples visibly compromised by haemolysis, hyperlipidemia, or bacteraemia.

Specimens should be free of microbial contamination and may be stored at 2–8 °C for 7 days. Fresh specimens may be stored long-term (3 months) at –20 °C (or lower). One freeze/thaw cycle of the specimens or 30 min heat inactivation at 56 °C does not affect the test result. Not less than 20 µl of serum should be used as a sample.

7 Assay procedure

7.1 Reagent preparation

Before beginning the assay reagents and samples should be at room temperature (20-25°C) for approximately 30 minutes and can remain at room temperature during testing.

It is recommended to perform the assay using at least 2 strips.

All bottles to be used for preparation of reagents must be cleaned thoroughly and rinsed with distilled or de-ionised water before use.

7.1.1 Preparation of wash buffer solution

If the vial with wash buffer concentrate contains salt sediment, keep the vial with the concentrate at (37 ± 1) °C until complete dissolution of the salts.

Transfer the content of one vial with wash buffer concentrate into a 1-l graduated cylinder and adjust the volume to 650 ml with distilled water.

When using one or several strips of the plate, take the corresponding amounts of wash buffer concentrate and distilled water necessary for ELISA (see Table 1).

Store unused wash buffer concentrate at 2–8°C during the shelf life; wash buffer solution at 2–8°C for 1 month.

7.1.2 Preparation of other buffers

Serum dilution buffer, serum preliminary dilution buffer, conjugate dilution buffer, and substrate buffer are ready for use.

Possibly there will be sediment in vials with serum dilution buffer and conjugate dilution buffer. Stir thoroughly before use.

When using one or several strips of the plate, transfer the necessary amounts of serum dilution buffer and serum preliminary dilution buffer into clean vials (see Table 1).

Store unused serum dilution buffer and serum preliminary dilution buffer during the kit shelf life at 2–8°C.

7.1.3 Preparation of control samples

Negative control and positive control are ready for use.

After opening the vial, store the control samples for 48 hours at 20–25°C; during the kit shelf life - at 2–8°C.

7.1.4 Preparation of Calibrator

Dissolve calibrator in 200 µl of distilled water 30–40 min before performing the assay.

Dissolve the restored calibrator in serum preliminary dilution buffer at a ratio of 1:10 using a plate for preliminary dilution. Add 90 µl of serum preliminary dilution buffer to each well of the plate for preliminary dilution and supplement with 10 µl of restored

calibrator. Stir the solution five times by pipetting; the serum preliminary dilution buffer should change the color from yellow-orange to crimson.

Store the restored calibrator at 2–8°C for 1 month; at –20°C for 6 months; freezing–thawing is permitted up to three times.

7.1.5 Preparation of conjugate working solution

Prepare the solution 5 min before adding to the plate wells.

Take the volume indicated on the label (V is conjugate volume in ml) from the vial with conjugate and transfer to the vial with conjugate dilution buffer. Mix the solution thoroughly avoiding foaming.

When using one or several strips of the plate, transfer conjugate dilution buffer into a clean vial and add conjugate in amounts necessary for ELISA (see Table 1).

Store conjugate working solution for 15 min at 20 – 25°C. When using one or several strips of the plate, store the remaining conjugate concentrate during the kit shelf life at 2–8°C.

7.1.6 Preparation of TMB chromogen working solution

Prepare TMB chromogen working solution 5 min before use in a place absent of straight sunlight.

Take the volume indicated on the label (V is TMB chromogen volume in ml) from the vial with TMB chromogen and transfer to the vial with substrate buffer. Mix the solution carefully.

When using one or several strips of the plate, transfer substrate buffer and TMB chromogen into a clean vial in the amounts necessary for the assay (see Table 1).

Store the prepared TMB chromogen working solution up to 20 min at 20–25°C protected from light.

7.1.7 Preparation of stop solution

Stop solution is ready for use.

When using one or several strips of the plate, transfer the stop solution in a clean vial in amount necessary for the assay (see Table 1).

Storage is unlimited.

Table 1. Necessary amounts of Measles–IgG–DS components

		Number of used strips											
		1	2	3	4	5	6	7	8	9	10	11	12
Volume, ml	Wash buffer concentrate	2	4	6	8	10	12	14	16	18	20	22	24
	Water	To 50	To 100	To 150	To 200	To 250	To 300	To 350	To 400	To 450	To 500	To 550	To 600
	Serum dilution buffer, serum preliminary dilution buffer, conjugate dilution buffer	1	2	3	4	5	6	7	8	9	10	11	12
	Substrate buffer	1	2	3	4	5	6	7	8	9	10	11	13
	Conjugate, TMB chromogen	1/12*V*	1/6*V*	1/4*V*	1/3*V*	5/12 *V*	1/2*V*	7/12*V*	2/3*V*	3/4*V*	5/6*V*	11/12*V*	*V*
	Stop solution	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6

7.2 Preparation of ELISA strips

Bring plastic pack with strips to room temperature (20-25 °C) before opening to prevent condensation on the ELISA plate. After the plastic pack has been opened, the ELISA strips are stable for 4 weeks at 2–8 °C if repacked thoroughly.

7.3 Preparation of sera

Human blood sera (plasma), which is fresh, or stored for 7 days at 2–8°C, or stored for 3 months at –20°C are used to detect and quantitatively determine human IgG antibodies to measles virus.

- To exclude false positives, the studied samples should be prepared and stored under sterile conditions excluding any possibility of bacterial contamination. Refine the serum samples containing sediment or aggregates by centrifugation. Never use serum samples with evident bacteremia. Avoid repeated cycles of freezing–thawing the samples. Never assay a pool containing several samples. Take each serum sample and solution with a new tip.
- Before assaying, dilute each serum sample with serum preliminary dilution buffer at a ratio of 1:10 using the plate for preliminary serum dilution: add to each well of the plate 90 µl of serum preliminary dilution buffer and supplement 10 µl of sera to be assayed. Mix the solution five times by pipetting; the serum preliminary dilution buffer color should change from yellow-orange to crimson.
  Store the diluted sera up to 2 hours at 20–25°C.

7.4 Procedure notes

- Do not perform the test in the presence of reactive vapours (e.g. from sodium hypocloride, acids, alkalis, or aldehydes) or dust because the enzymatic activity of the conjugate may be affected.
- The kit contains two functionally different plates with visually similar strips. Do not mistake strips.
- Before testing begins inspect the plate coated with antigen and ensure that it’s secure. Strips should be handled with care.
- ELISA strips may be used only once.
- All reagents and samples must be mixed well before use.
- To avoid contamination, do not touch the top or the bottom of the plates, the edge of the wells or the liquid in the wells with fingers or pipette tips. Use fresh tips when put samples or solutions in the wells.
- All pipetting steps should be performed with the utmost care and accuracy. Cross-contamination between reagents and samples will invalidate results. Avoid microbial or any other contamination of reagents.
- Remove any bubbles in the wells, e.g. by gentle tapping.
- Prevent evaporation during sample incubation (e.g. by covering the plates with sealer).
- Routine maintenance of aspiration/wash system is strongly recommended to prevent carryover from highly reactive samples to nonreactive ones.
- Do not use components from test kits of different lots or mix them during the process of solution preparation.

7.5 Wash procedure

- Incomplete washing will adversely affect the test outcome.
- Aspirate the well contents completely into a waste flask. Then fill the wells completely with wash buffer avoiding overflow of buffer of one well to another and allow soaking (approx. 30 seconds).
- Aspirate completely and repeat the wash and soak procedure four additional times for a total of five washes.
- Make sure that no fluid remains on the top and the bottom of the strips after the last aspiration (e.g. by blotting with absorbent tissue).
- If you don't have a micro well aspiration/wash system, remove wash buffer from the wells by active shaking the strips against an absorbent tissue (for example a multiply folded filter paper).

7.6 Immunoassay procedure

Prepare dilutions of calibrator and assayed samples in serum preliminary dilution buffer , as described above, using the plate for preliminary dilution.

Open the vials with negative control and positive control.

Note! *All the procedures described in items 7.6.1-7.6.10 should be prepared using the plate coated with antigen*

7.6.1 Wash the plate coated with antigen once with wash buffer solution, adding 200–250 µl of buffer into each well.

Note! *In order to prevent an immunosorbent desiccation addition of the controls and assayed samples to the plate should not exceed 10 min.*

7.6.2 Add 100 µl of positive control to well A1 and 100 µl of negative control to wells B1 and C1 each of the plate coated with antigen. Add 100 µl of serum dilution buffer to well D1 for the control of conjugate.

7.6.3 Add 90 µl of serum dilution buffer to the rest of the wells. Add 10 µl of calibrator working solution to the well E1 and two last wells in the particular assay. Add 10 µl of the assayed sera preliminary diluted tenfold to the rest of the wells of the plate coated with antigen (thus, final dilutions of restored calibrator and sera are 100-fold). Mix the solutions five times by pipetting; serum dilution buffer solution should be crimson color.

7.6.4 Close the plate with the lid or seal with adhesive tape and incubate for 30 min at (37±1) °C.

7.6.5 After incubation, wash the plate coated with antigen with wash buffer solution five times, as described above.

7.6.6 After washing and removal of the residual moisture, add 100 µl of conjugate working solution to each well. Close the plate coated with antigen with the lid or seal with adhesive tape and incubate for 30 min at (37±1) °C.

Note! *Addition of conjugate working solution to the plate coated with antigen should not exceed 5-7 min.*

7.6.7 After incubation, wash the plate coated with antigen with Wash buffer solution five times, as described above.

7.6.8 Add 100 µl of TMB chromogen working solution. Close the plate coated with antigen with the lid or seal with adhesive tape and keep for 20 min in a shaded place at 20–25°C.

7.6.9 Stop the reaction by supplementing each well with 50 µl of stop solution.

7.6.10 Blank the reader on air (without strips) and read the absorbency of the solutions in each well at 450 nm.

8 Results

8.1 Quality control

The results are considered significant only if optical density (OD) in the conjugate control well (**$OD_{conjugate}$**) does not exceed 0.1; average OD value for negative control wells (**OD_{C-}m**) does not exceed 0.2; and OD value for positive control well (**OD_{C+}**) is at least 1.0.

ODcritical is calculated as

ODcritical = (**OD_{C-}m**) + 0.2

The test is positive if OD of the serum assayed is higher than (**ODcritica**l + 0.05).

The test is negative if OD of the serum assayed is lower than (**ODcritica**l - 0.05).

The interval between (**ODcritica**l $\pm$ 0.05) = **"cut-off"**.

If OD of the assayed serum is in the range **"cut-off"**—the test result is classed as equivocal. In such cases follow-up testing is advisable. The sample is assayed again using a 50-fold dilution. If the second test is positive, another sample from the same person is assayed. If the second test is equivocal, the sera should be assayed by other methods.

8.2 Calculation of sera specific activity

For the positive sera, specific activity in IU/ml is calculated. If OD exceeds 2.0, specific activity is calculated only after the second assay with the same sample diluted 1:400 and 1:800. There are 2 steps of calculations:

8.2.1 Calculate correction coefficient as the ratio of activity of the calibrator indicated in Certificate of Analysis over OD mean of calibrator and multiply it by patient serum OD. The resulting formula is:

$$\mathrm{Pser} = \mathrm{ODser} \times \frac{\mathrm{A\ Cr}}{\mathrm{OD_{m\,Cr}}}$$

where

Pser is product of the OD serum assayed on the correction coefficient;

ODser is OD value of the assayed serum;

A Cr is the value of calibrator specific activity (indicated in Certificate of Analysis); and

$OD_{m\ Cr}$ is the arithmetic mean of calibrator OD values.

8.2.2 For calculation of Aser in IU/ml use the formula:

$$Aser = 10^{(Pser-a)/b}$$

where **a** and **b** are the constants given in Certificate of Analysis and specific for each lot.

Table 2. Calculation example (OU, optical units)

$OD_{conjugate}$ value (well E1), OU	0.081 < 0.1
OD_{C+} value (well A1) , OU	2.661 > 1.0
OD_{C-} values (well B1 and C1), OU	0.123; 0.125 < 0.2
The values given above demonstrate that ELISA results are significant	
Mean OD_{C-}m value , OU	(0.123 + 0.125)/2 = 0.124
OD critical value, OU	0.2 + 0.124 = 0.324
«Equivocal» result zone, OU	0.324 ± 0.05 = [0.274; 0.374]
$OD_{m\ Cr}$ value, OU	(0.573 + 0.563 + 0.553)/3 = 0.563
A Cr value according to Certificate of Analysis, IU/ml	0.83
ODser value, OU	0.754
Calculation of **Pser**	0.754 × 0.83/0.563 = 1.112
Estimation of **Aser** according to the plot in Certificate of Analysis**:** Aser = **1.2 IU/ml**	
a value according to Certificate of Analysis	0.7
b value according to Certificate of Analysis	8.57
Calculation of (**Pser** - **a**)/**b**	(1.112-0.7)/8.57=0.048
Calculation of **Aser**	$10^{0.048}$**=1.117 IU/ml**

Note: *Use our software for assay quality control and calculating the sera specific activity. This product is free on* http://www.mbunion.com

8.3 Interpretation of the results

Determinations used to assess significant changes in activity should always be performed in the same run and in the same test dilution. In these cases a difference of more then a factor of 2 is indicative of such a change. When comparing results from different runs, identical lots of reagent must be used and the test samples must be assessed in the same dilutions. Under this conditions differences of more than a factor of 3 indicate the significant change in activity.

A negative classification signifies that no virus-specific IgG can be detected. If exposure to the virus is suspected despite a negative finding, a second sample should be collected no less than 2 to 3 weeks after the suspected time of virus exposure and should be tested together with the first sample. Seroconversion from negative in the first sample to positive in the second sample is evidence of a recent infection, or of a successful vaccination, or of the administration of immune globulin, such as is recommended for recent measles infection in HIV-positive children.

A positive sample classification means that anti-measles IgG has been detected. If no concurrent anti-measles IgM is detected, in Measles–IgM–DS test it can be assumed that the patient had been infected with measles in the past, had been vaccinated against measles virus or have received immunoglobulin.

Detection of single virus-specific antibody activity, even a high activity, does not provide a proof of a recent infection; there are no normal values which can be used as

reference values. Nevertheless, for a wide range of investigative purposes, the quantitative evaluation remains an indispensable diagnostic tool.

9 The form of manufacturing

Measles–IgG–DS is manufactured as a kit packed in a rigid box. Instruction manual is provided inside the box. The box is covered with a shrinkable film.

Measles–IgG–DS contains the following components:

1	ELISA 96-well stripped plate in a pack sealed underpressure or containing silica gel	1 block	
2	The plate for preliminary serum dilution	1 block	
3	Wash buffer concentrate	1 vial	26.0 ml
4	Serum dilution buffer	1 vial	12.0 ml
5	Serum preliminary dilution buffer	1 vial	12.0 ml
6	Conjugate dilution buffer	1 vial	12.0 ml
7	Substrate buffer	1 vial	13.0 ml
8	Positive control	1 vial	2.0 ml
9	Negative control	1 vial	3.0 ml
10	TMB chromogen	1 vial	0.5 ml
11	Calibrator	1 vial	
12	Conjugate	1 vial	0.5 ml
13	Stop Solution	1 vial	6.0 ml

10 Shelf life, storage and transportation conditions

Shelf life of Measles–IgG–DS is 6 months.
Store at 2 - 8°C in the dry dark place.
Do not freeze the kit.
Transport the kit at 2 - 8°C; transportation is allowed up to 3 days at the temperature not exceeding 25°C.
Do not freeze the kit.

11 Related products

CAT #	PRODUCT NAME	DESCRIPTION	PACK SIZE
D-402	Measles-IgG-DS	ELISA kit for detection and quantitative determination of IgG antibodies to measles virus in human serum or plasma and for aid in the diagnosis of patients suspected of having measles and in the estimation of the vaccination anti-measles success.	(12x8) 96 tests
D-403	Measles-IgM-DS	ELISA kit for detection of IgM antibodies to measles virus in human serum or plasma and is indicated for aid in the diagnosis of patients suspected of having measles.	(12x8) 96 tests

12 References

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