



VIM IgM (Human) ELISA Kit

Catalog Number KA1476

96 assays

Version: 02

Intended for research use only

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Introduction

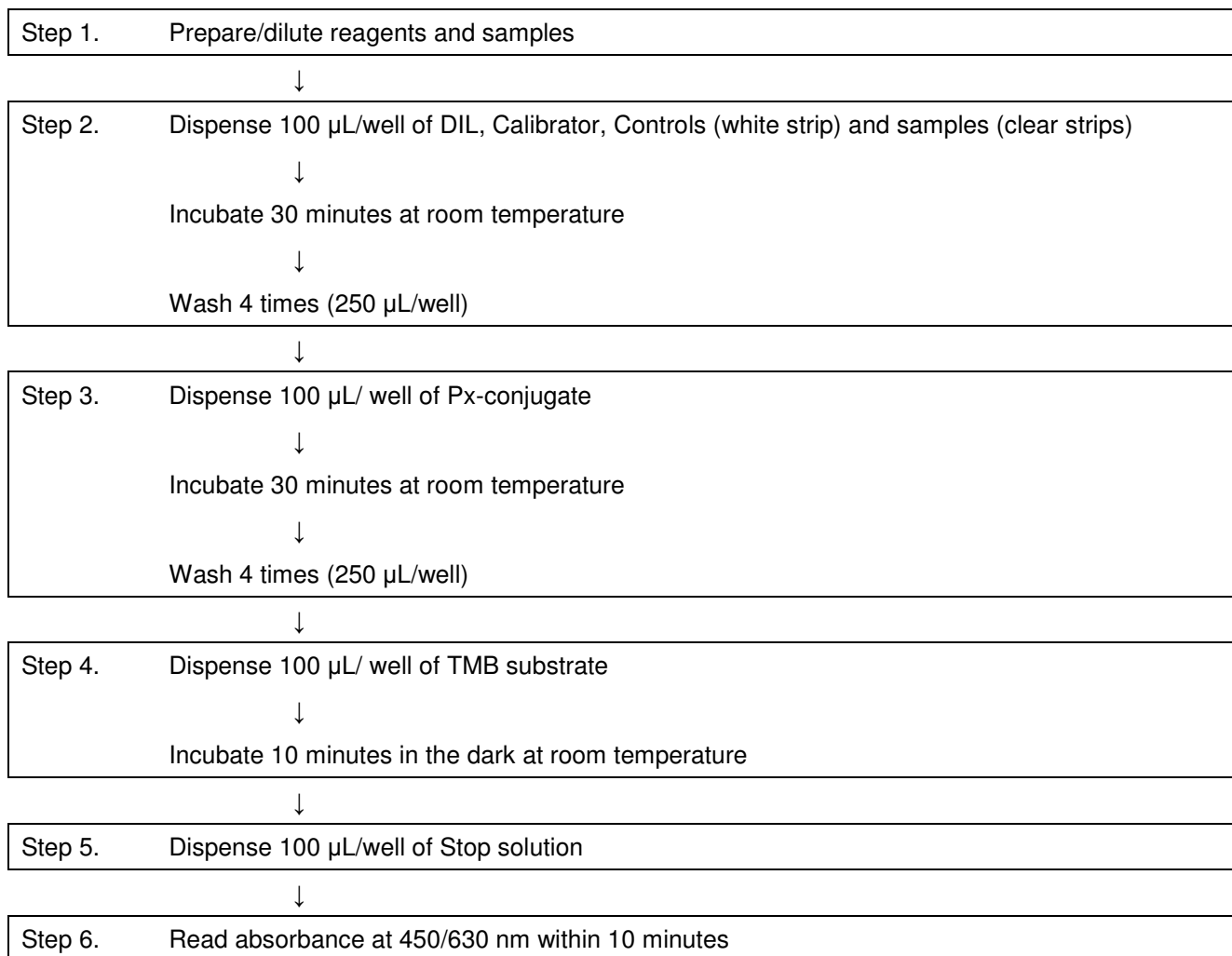
Intended Use

Anti-VIM IgM (Human) ELISA Kit is an ELISA kit for a semiquantitative detection of human IgM antibodies to the intermediate filament protein vimentin in human serum.

Principle of the Assay

Anti-VIM IgM (Human) ELISA Kit is a solid-phase immunoassay. Polystyrene strips are coated with vimentin, purified Ecoli expressed recombinant protein. Anti-vimentin antibodies in serum samples bind to the immobilized antigens. The antibodies that do not bind are washed away and those that formed complexes with the antigens are later on recognised by animal anti-human IgM antibodies labelled with horseradish peroxidase. The presence of labelled antibodies is revealed by an enzymatic reaction with a chromogenic substrate. Negative sera do not react and the mild change in colour, if present, may be attributed to the reaction background.

Flow Chart



General Information

Materials Supplied

List of component

Component	Amount
ELISA break-away strips (clear) coated with specific antigen	96 wells
ELISA break-away strips (green) coated with control antigens (calibration strips)	48 wells
Negative control, r.t.u. ¹⁾	1.3 mL
Positive control, r.t.u. ¹⁾	1.3 mL
Calibrator, r.t.u. ¹⁾	1.3 mL x 2
Anti-human IgM antibodies labelled with horseradish peroxidase, (Px-conjugate), r.t.u.	10 mL x 2
Wash buffer concentrate, 10x concentrated	125 mL
Dilution buffer, r.t.u.	125 mL
Chromogenic substrate (TMB substrate), r.t.u.	13 mL x 2
Stop solution, r.t.u.	30 mL
Sealable pouch for unused strips	1 bag

¹⁾ r.t.u = ready to use

Storage Instruction

- ✓ Store the kit and the kit reagents at 2 to 10°C, in a dry place and protected from the light. Store unused strips in the sealable pouch and keep the desiccant inside.
- ✓ Store serum samples at 2 to 10°C up to one week. For longer period make aliquots and keep them at -20°C. Avoid repeated thawing and freezing.
- ✓ Do not store diluted samples. Always prepare fresh.
- ✓ Kits are shipped in cooling bags, the transport time up to 72 hours have no influence on expiration. If you find damage at any part of the kit, please inform the manufacturer immediately.
- ✓ Expiration date is indicated at the ELISA kit label and at all reagent labels.

Materials Required but Not Supplied

- ✓ Distilled or deionised water for dilution of the Wash buffer concentrate.
- ✓ Appropriate equipment for pipetting, liquid dispensing and washing.
- ✓ Spectrophotometer/colorimeter (microplate reader – wavelength 450 nm).

Precautions for Use

- Safety Precautions

- ✓ All ingredients of the kit are intended for laboratory use only.
 - ✓ Controls and Calibrator contain human sera that has been tested negative for HBsAg, anti-HIV-1,2 and anti-HCV. However they should be regarded as contagious and handled and disposed of according to the appropriate regulations.
 - ✓ Autoclave all reusable materials that were in contact with human samples for 1 hour at 121 °C, burn disposable ignitable materials, decontaminate liquid wastes and nonignitable materials with 3% chloramine. Liquid wastes containing acid (Stop solution) should be neutralized in 4% sodium bicarbonate solution.
 - ✓ Handle Stop solution with care. Avoid contact with skin or mucous membranes. In case of contact with skin, rinse immediately with plenty of water and seek medical advice.
 - ✓ The waste from strip washing disinfect in waste container using appropriate disinfection solution (e.g. Incidur, Incidin, Chloramin, ...) in concentrations recommended by the producer.
 - ✓ Do not smoke, eat or drink during work. Do not pipette by mouth. Wear disposable gloves while handling reagents or samples and wash your hands thoroughly afterwards. Avoid spilling or producing aerosol.
- Handling Precautions
 - ✓ Manufacturer guarantees performance of the entire ELISA kit.
 - ✓ Follow the assay procedure indicated in the Instruction manual.
 - ✓ Calibrator, Dilution buffer and Px-conjugate contain preservative ProClin 300®.
 - ✓ Avoid microbial contamination of serum samples and kit reagents.
 - ✓ Avoid cross-contamination of reagents.
 - ✓ Avoid contact of the TMB substrate with oxidizing agents or metal surfaces.
 - ✓ Variations in test results are usually due to:
 - * Insufficient mixing of reagents and samples
 - * Inaccurate pipetting and inadequate incubation times
 - * Poor washing technique or spilling the rim of well with sample or Peroxidase conjugate
 - * Use of identical pipette tip for different solutions

Assay Protocol

Reagent Preparation

- ✓ Allow all kit components to reach room temperature.
- ✓ Vortex samples, Calibrator and the controls in order to ensure homogeneity and mix all solutions well prior use.
- ✓ Dilute serum samples 1:100 in Dilution buffer and mix (*e.g. 5 µL of serum sample + 500 µL of Dilution buffer*). Do not dilute the controls and Calibrator, they are ready to use.
- ✓ Prepare Wash buffer by diluting the Wash buffer concentrate 10 times with an appropriate volume of distilled or deionised water (*e.g. 100 mL of the concentrated Wash buffer + 900 mL of distilled water*). If there are crystals of salt present in the concentrated Wash buffer, warm up the vial to 32 to 37°C in a water bath. Diluted Wash buffer is stable for one week if stored at 2 to 10°C.
- ✓ Do not dilute Px-conjugate, TMB substrate and Stop solution, they are ready to use.

Assay Procedure

1. Allow the microwell strips sealed inside the aluminium bag to reach room temperature. Withdraw one Calibration strip and an adequate number of strips with specific antigen. Return any unused strips into the provided pouch and seal them carefully with the desiccant kept inside.
2. Pipette 100 µL of Dilution buffer, Calibrator, Positive and Negative Control (white calibration strips) and diluted samples (S1, S2, S3, ...) (clear strips) according to the Figure. 1 (Pipetting scheme). It is sufficient to apply serum samples as singlets, however, if you wish to minimise the laboratory error apply the samples in doublets.
3. Incubate 30 (+/- 2 minutes) at room temperature.
4. Aspirate the liquid from wells into a waste bottle containing an appropriate disinfectant (see Safety Precautions). Wash and aspirate the wells four times (4x) with 250 µL/well of Wash buffer. Avoid cross-contamination between wells! If any liquid stays trapped inside the wells, invert the plate and tap it on an adsorbent paper to remove the remaining drops.
5. Mix Px- conjugate r.t.u. well and then add 100 µL of the Px-conjugate r.t.u. into each well (clear and white strips).
6. Incubate 30 minutes (+/- 2 minutes) at room temperature.
7. Aspirate and wash four times (4x) with 250 µL of Wash buffer (as in step 4).
8. Dispense 100 µL of TMB substrate into each well.
9. Incubate for 10 minutes (+/-5 seconds) at room temperature.
The time measurement must be started at the beginning of TMB dispensing. Cover the strips with an aluminium foil or keep them in the dark during the incubation with TMB substrate.
10. Stop the reaction by adding 100 µL of Stop solution. Tap gently the microplate few times to ensure complete mixing of the reagents.

11. Measure the absorbance at 450 nm with a microplate reader within 10 minutes. It is recommended to use a reference reading at 620 - 690 nm.

Data Analysis

Calculation of Results

- Processing of Results
1. Begin the processing with subtraction of the absorbance of the DIL well (background absorbance) from the absorbances Calibrator and controls and the absorbance of DIL well in testing strips from all values of the samples.
 2. Compute the Calibrator mean absorbance from the three Calibrator wells. If any of the three Calibrator absorbances is different from the mean in more than 20% then exclude the deviating well from the calculation and compute a new Calibrator mean using the values from the other two wells.
 3. Compute the cut-off value by multiplying the Calibrator mean with a Correction factor. The Correction factor is determined for each kit and it is indicated in the corresponding Instruction manual of the respective Lot.
 4. Compute the Positivity Index for each sample according to the following formula:

$$\text{Sample positivity Index} = \frac{\text{Sample absorbance}}{\text{Cut-off value}}$$

5. Express a serum reactivity according to Table 1 (Semiquantitative interpretation of results) – Index value ranges may differ between kits. The Lot relevant Result evaluation is indicated in the Instruction manual of the corresponding Lot.

Table 1. Semiquantitative interpretation of results:

Index value	Classification
< 1.00	negative
1.00 – 1.35	+/-
> 1.35	positive

Note! An indifferent sample reactivity, i.e. interpreted as +/-, requires repetition of the sample test. If the result is again indifferent then it is recommended to use an alternative testing method or to obtain another sample from the patient, usually withdrawn 1-2 weeks later.

Example of calculation:

Calibrator absorbances: = 0.961; 0.987; 0.954

Calibrator mean: = 0.967

Sample absorbance: = 0.800

Correction factor: = 0.27

Cut off value: = 0.967*0.27 = 0.261

Sample Positivity Index: = 0.800/0.261 = 3.07

Performance Characteristics

- Validity of the test
- ✓ The background absorbance in calibration strip (the absorbance of the DIL well) is less than 0.200. The background absorbance in strip with samples is less than 0.100.
- ✓ The absorbance of Negative Control is less than 0.250.
- ✓ The absorbance of Positive Control is higher than 1.500.
- ✓ Tolerated value of Calibrator is indicated in Quality control certificate.

Plate Layout

	1	Dilution Buffer	Calibrator	Calibrator	Calibrator	Negative control	Negative control	Positive control	Positive control
	2	Dilution Buffer	Sample 1	Sample 2	Sample 3	Sample...			
	3								
	4								
	5								
	6								
	7								
	8								
	9								
	10								
	11								
	12								
A									
B									
C									
D									
E									
F									
G									
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