

Instructions For Use

Serazym[®] Human IgM








REF E-004
96



IVD

In-vitro-diagnostic-device

Enzyme immunoassay for determination of
human IgM in human serum samples
or culture supernatants

| | | | |
|---|------------------------------------|---|-----------------------------|
| REF | Catalogue- No. | LOT | Lot-No. |
|  | Storage temperature |  | Manufacturer |
|  | Notice advices |  | Use by |
|  | Consult instructions for use |  | Number of Determinations |
| | |  | Biohazard |

Introduction

Human immunoglobulin M (IgM) is characterized by its pentameric structure with a molecular weight of 971,000. It is synthesized by B-lymphocytes with an average synthesis rate of 6.7 mg per kg body weight per day and has a half life of 5 days. Due to its high molecular weight IgM predominantly (75-80 %) occurs in the intravascular space.

IgM molecules represent the phylogenetically oldest immunoglobulin class. They are often poly-reactive and produced as the primary B-cell reaction to antigenic stimuli. During B-cell ontogenesis monomeric IgM appears as first B-cell receptor (sIgM).

IgM molecules bind complement much more effectively than IgG and strongly enhance phagocytosis additionally to their agglutinating and precipitating properties. Fetal IgM is produced from the 20th week of pregnancy. IgM concentrations exceeding 0.2 g/l in the blood of the umbilical cord are evident for an intra-uterine infection.

The IgM concentration in neonates makes up only 10 % of adult values but it increases up to the second year of age.

IgM serum concentrations are usually determined by nephelometry or turbidimetry. However these methods lack sensitivity concerning the monitoring of B-cell function (immunoglobulin production and secretion) in lymphocyte cultures e.g. for differential diagnosis of primary antibody deficiencies or estimation of the severity of secondary antibody deficiencies.

References: (1) Thomas L. Labor und Diagnose. Frankfurt: TH Books Verlagsgesellschaft, 5 Auflage 1998

Intended Use

Serazym[®] Human IgM is an *in-vitro* diagnostic test for determination of human IgM in human serum samples or culture supernatants.

Principle Of The Test

Serazym[®] Human IgM is an enzyme immunoassay for quantitative determination of human IgM in human serum samples or culture supernatants.

In the first incubation step diluted samples, ready-to-use calibrators and control react with the solid-phase adsorbed anti-human IgG-antibodies (sheep). After 30 minutes at room temperature unbound components are removed by a washing step.

In the second incubation step bound human IgM reacts with horseradish peroxidase (HRP)-labelled anti-human-IgM antibodies (sheep) and after 30 minutes at room temperature unbound conjugate is removed by a washing step.

In the third step the HRP converts the subsequently added colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) within a 15 min reaction time at room temperature into a blue product. The enzyme reaction is terminated by sulphuric acid dispensed into the wells turning the solution from blue to yellow.

The absorbance of the solution read at 450/620-690 nm is directly proportional to the specifically bound concentration of human IgM in the unknown sample.

A reference curve is created from the absorbance values of the calibrators. The absorbance values of the unknown serum samples are transformed into their corresponding IgM concentrations by reading from the reference curve.

Preparation And Storage Of Samples

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Lipaemic, hemolytic and contaminated samples should not be used.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at -20 °C.

Specimen preparation

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Note: Samples have to be diluted 1+2,500 in two steps

Step 1: 10 µl sample + 490 µl sample diluent (3)
Step 2: 10 µl from step 1 + 490 µl sample diluent (3)

The samples may be kept at 2...8 °C for up to two days. Long-term storage requires -20 °C.

Diluted samples should be investigated at the same day of dilution.

Test Components For 96 Wells

| | | |
|--------------------------------------|--|---|
| 1 WELLS | Microtitration plate 12 single breakable 8-well strips (in all 96 wells) coated with anti-human-IgM (sheep) | 1 vacuum-sealed with desiccant |
| 2 WASHBUF CONC 100X | Concentrated Wash buffer, 100-fold for 2,000 ml solution | 20 ml concentrate white cap |
| 3 DIL | Sample diluent | 2 X 100 ml ready-to-use black cap |
| 4 CAL 1-5 | Calibrators 1 - 5 (diluted serum) CAL 1 = 20 ng/ml CAL 2 = 75 ng/ml CAL 3 = 300 ng/ml CAL 4 = 600 ng/ml CAL 5 = 1,200 ng/ml | 1.0 ml ready-to-use white cap |
| 5 CONTROL | Control (diluted serum) 150 ng/ml | 1.0 ml ready-to-use green cap |
| 6 CONJ HRP | Conjugate anti-human-IgM antibodies (sheep),labelled with HRP | 15 ml ready-to-use red 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 |

Materials Required But Not Provided

- Adjustable one channel micropipette 0.100 – 1.000 ml and 0.010 – 0.100 ml
- Adjustable 8-channel micropipette 0.050 – 0.200 ml
- Pipette Tips
- Graduated measuring flasks 10 ml and 1000 ml
- Microtitration plate washer (automatic or hand wash head)
- Microtitration plate reader with 450 nm and 620 - 690 nm filters
- Distilled or de-ionized water

Preparation And Storage Of Reagents

Kit size and expiry

The *Serazym*[®] Human IgM has been designed for 96 determinations.

The complete kit with unopened reagent bottles and microtitration strips is stable until the expiry date printed on the kit box in case of storage at 2...8 °C. Once opened all kit components are stable for up to 1 month under appropriate storage conditions (2...8 °C). When stored at 2...8 °C the diluted ready-to-use wash solution is stable for up to 1 month.

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. 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 **WASHBUF CONC 100X (2)** 100 times (1 +99) with de-ionized or distilled water.
For example: 5 ml **WASHBUF CONC 100X (2)** + 495 ml distilled water.

Make sure the soaking time of the wash solution in the wells is at least 5 seconds per wash cycle and that the remaining fluid is completely drained in every wash cycle.

Avoid exposure of the TMB substrate solution to light!

Assay Procedure

- Dilute specimens with sample diluent (3) 1 + 2,500 (v/v) in two steps as described above.
- Avoid any time shift during dispensing of reagents and samples.

Working steps

1. Warm all reagents to room temperature (20...25 °C) before use. Mix gently without causing foam.
2. Dispense
100 µl CAL 1 - 5 (ready-to-use calibrators 1, 2, 3, 4, 5) **(4)**,
100 µl CONTROL (ready-to-use control) **(5)** and
100 µl diluted samples resp.
into the intended wells.
3. Cover plate, incubate **30 min** at room temperature.
4. Decant, then wash wells **three** times using **300 µl** wash solution (prepared from **(2)**).
5. Add **100 µl CONJ HRP (6)** to each well.
6. Cover plate, incubate **30 min** at room temperature.
7. Decant, then wash wells **three** times using **300 µl** wash solution (prepared from **(2)**).
8. Add **100 µl SUBSTR TMB (7)** to each well.
9. Incubate **15 min** at room temperature protected from light.
10. Add **100 µl STOP (8)** to each well and mix gently.
11. Read absorbances at **450 nm** versus 620 - 690 nm within 30 min after reaction stop.

Result Interpretation

Create a reference curve by plotting the mean absorbance values of the calibrators CAL 1-5 (Y-axis) to the corresponding human-IgM concentrations (X-axis).

Determine the human-IgM-concentrations of the unknown samples by referring their mean absorbances to the reference curve and multiply with the dilution factor.

Test validity

The test run is valid if:

- absorbance of calibrator CAL 1 is ≤ 0.5 ,
- absorbance of calibrator CAL 5 is ≥ 1.2 and
- the control is determined between 120 and 180 ng/ml.

If the above mentioned quality criteria are not met, repeat the test and make sure the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash Steps etc.). In case of repeated failure of the quality criteria contact your supplier.

Reference Values (human serum samples)

| Human IgM | |
|-------------------------|--|
| Normal range (1) | |
| Neonates | 100 – 300 µg/ml |
| Children | |
| 1-12 months | 100 – 1,000 µg/ml |
| 1-13 years | 300 – 1,500 µg/ml |
| Adults | 400 – 2,300 µg/ml |
| Increased | Proliferative monoclonal gammopathy (Morbus Waldenström), Acute and chronic infections, Autoimmune diseases, Primary biliary cirrhosis, Chronic destructive cholangitis |
| Decreased | Nephrotic syndrome, Inherited and acquired Defect immunopathy, Exsudative enteropathy |

Performance Characteristics

Precision

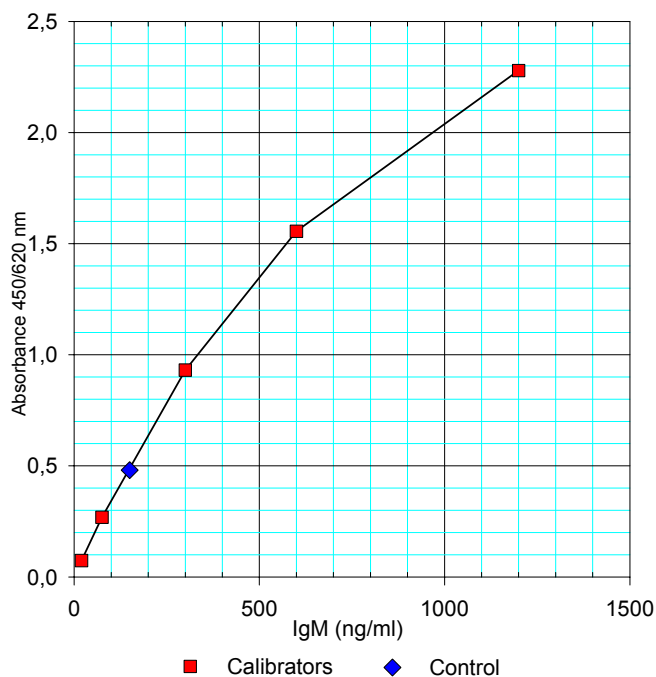
Intra-Assay coefficient of variation (CV %)

| sample | Mean concentrations | CV (%) |
|---------|---------------------|--------|
| Serum 1 | 1 215 µg/ml | 2.87 |
| Serum 2 | 1 119 µg/ml | 4.11 |
| Serum 3 | 1 059 µg/ml | 4.48 |
| Serum 4 | 793 µg/ml | 5.24 |

Inter-Assay coefficient of variation (CV %):

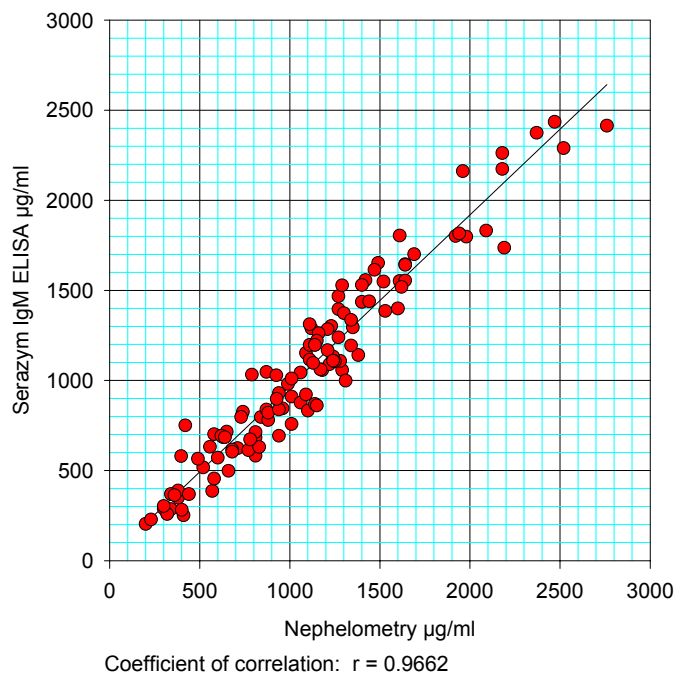
| sample | Mean concentrations | CV (%) |
|---------|---------------------|--------|
| Serum 1 | 1 301 µg/ml | 7.60 |
| Serum 2 | 1 123 µg/ml | 7.16 |
| Serum 3 | 1 002 µg/ml | 8.70 |
| Serum 4 | 820 µg/ml | 10.70 |

Typical reference curve



Method correlation

IgM determination of 120 human serum samples with Serazym ELISA and Nephelometry



Limitations of the method

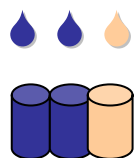
Sera of healthy individuals should be tested within the normal range in the Serazym[®] Human IgM, but apparently healthy persons can show increased or decreased serum IgM-concentrations.

It is recommended that each laboratory establishes its own normal and pathological reference ranges as usually done for other diagnostic parameters, too. Therefore the above mentioned reference values provide a guide only to values which might be expected.

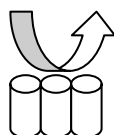
A final interpretation of the results should consider clinical findings as well.

Incubation Scheme

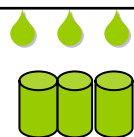
Serazym[®] Human IgM (E-004)



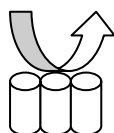
| | | |
|---------------|--|--------------------------------|
| 100 µl | | CAL 1-5 (4) |
| 100 µl | | CONTROL (5) and |
| 100 µl | | diluted sample resp. |
| 30 min | | incubation at room temperature |



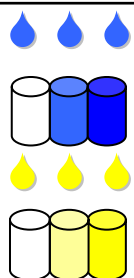
| | | |
|-----------------|--|--------------------|
| 3 x Wash | | with wash solution |
|-----------------|--|--------------------|



| | | |
|---------------|--|--------------------------------|
| 100 µl | | CONJ HRP (6) |
| 30 min | | incubation at room temperature |



| | | |
|-----------------|--|--------------------|
| 3 x Wash | | with wash solution |
|-----------------|--|--------------------|



| | | |
|---------------|--|---|
| 100 µl | | SUBSTR TMB (7) |
| 15 min | | incubation at room temperature protected from light |

| | | |
|-------------------------|--|-------------------|
| 100 µl | | STOP (8) |
| Read absorbances | | at 450/620-690 nm |

Common Advices and Precautions

This kit is for *in-vitro* use only. 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.

All reagents should be kept at 2...8 °C before use.

Some of the reagents contain small amounts of Thimerosal (< 0.1 % 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 patient 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.