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Instructions For Use

Serazym® Human IgA

REF E-007

96

IVD

In-vitro-diagnostic-device

Enzyme immunoassay for determination of human IgA in human serum samples or culture supernatant

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

Introduction

In human serum immunoglobulin A (IgA) predominantly (85-90%) occurs as monomeric molecule with a molecular weight of 160,000. As dimeric or polymeric molecule existing IgA additionally contains a J-chain. About 40 % of the IgA molecules are intra-vascular.
About 60 mg IgA per kg body weight per day is synthesized by plasma cells. Its half-life is 5.4 to 5.9 days.
IgA antibodies neutralize virus antigens and bacterial toxins and as aggregated molecules they activate complement via the alternative pathway. IgA is the predominant immunoglobulin in secretory fluids. Secretory IgA almost exclusively occurs as dimeric molecule containing a glycoprotein with a molecular weight of 70,000 (secretory component). Secretory IgA fulfils protective functions on mucous membranes.
IgA is unable to pass the placental barrier and is therefore absent in fetal blood. IgA serum concentrations in children reach adult levels in the 12th year of age.

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

Intended Use

Serazym® Human IgA is an in-*vitro* diagnostic test for determination of human IgA in human serum samples or culture supernatant.

Principle Of The Test

Serazym® Human IgA is an enzyme immunoassay for quantitative determination of human IgA in human serum samples or culture supernatant.

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

In the second incubation step bound human IgA reacts with horseradish peroxidase (HRP)-labelled anti-human-IgA 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 IgA 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 IgA 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+5,000 in two steps

Step 1: 10 µl sample + 490 µl sample diluent (3)
Step 2: 10 µl from step 1 + 990 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-IgA (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-IgA 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 IgA 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 + 5,000 (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-IgA concentrations (X-axis).
Determine the human-IgA-concentrations of the unknown samples by referring their mean absorbances to the reference curve and multiplicate 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 IgA	
Normal range (1)	
Neonates	**70- 940 µg/ml**
Children	
1-12 months	**100 - 1,310 µg/ml**
1-13 years	**19 - 395 µg/ml**
Adults	**700 - 4,000 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

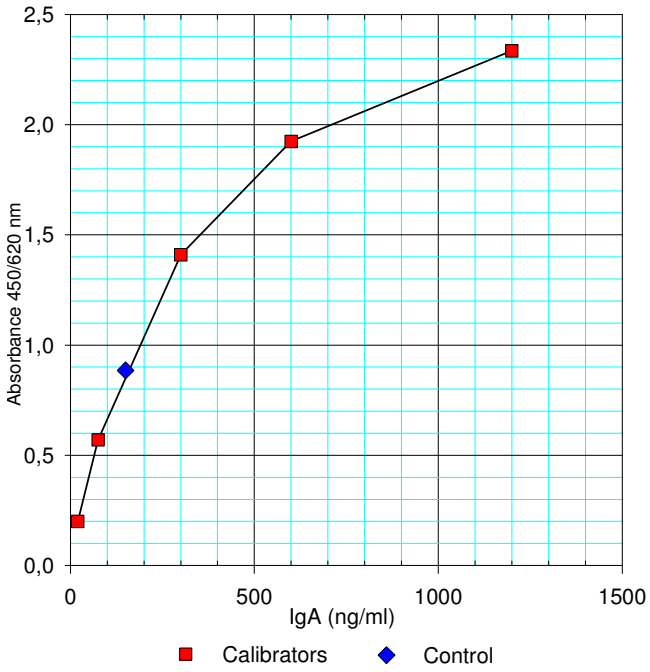
Intra-Assay coefficient of variation (CV %)

sample	**Mean concentrations**	CV (%)
Serum 1	1 702 µg/ml	4.55
Serum 2	1 679 µg/ml	2.89
Serum 3	1 588 µg/ml	2.69
Serum 4	1 323 µg/ml	2.14

Inter-Assay coefficient of variation (CV %):

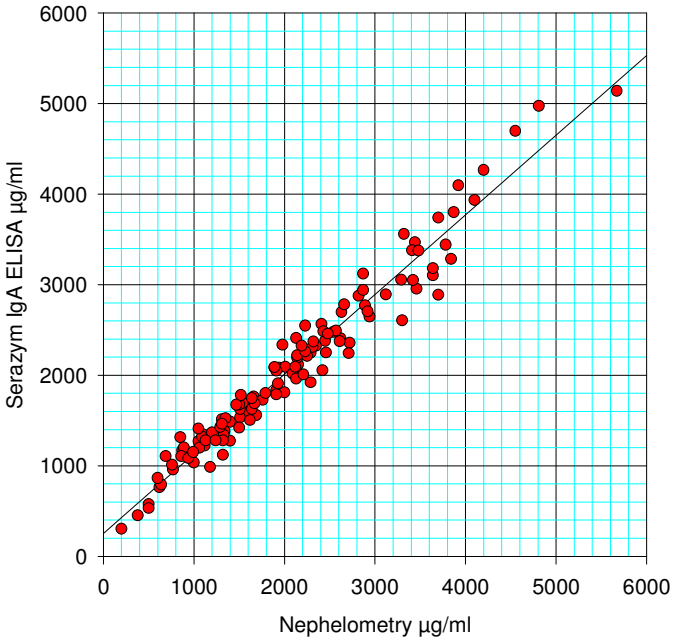
sample	**Mean concentrations**	CV (%)
Serum 1	1 924 µg/ml	6.96
Serum 2	1 794 µg/ml	4.46
Serum 3	1 659 µg/ml	4.50
Serum 4	1 258 µg/ml	4.86

Typical reference curve



Method correlation

IgA determination of 123 human serum samples with Serazym ELISA and Nephelometry



Coefficient of correlation: r = 0.9785

Limitations of the method

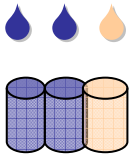
Sera of healthy individuals should be tested within the normal range in the *Serazym*® Human IgA, but apparently healthy persons can show increased or decreased serum IgA-concentrations.

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.

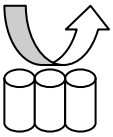
Incubation Scheme

Serazym® Human IgA (E-007)

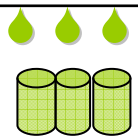


100 µl ***CAL 1-5 (4)***
100 µl ***CONTROL (5)*** **and**
100 µl **diluted sample resp.**

30 min incubation at room temperature

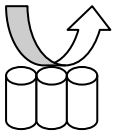


3 x Wash with wash solution

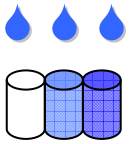


100 µl ***CONJ HRP (6)***

30 min incubation at room temperature

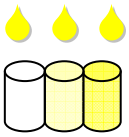


3 x Wash with wash solution



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.