DATENBLATT

Human IgG Subclass Elisa Combi Kit

For Quantification of Human Subclasses in Serum and Other Biological Fluids

Catalog #: CLBM1551

1 INTRODUCTION

The main interest in the determination of human IgG subclasses is based on the observation that they differ in biological properties. Variations in antigen recognition as well as differences in effector functions have been established (1, 2).
Studies have revealed that selective IgG subclass deficiencies are associated with susceptibility to viral or bacterial infections, indicative for a disturbed immune response (3).
The human IgG subclass ELISA combi kit is intended for measuring human IgG subclass levels in serum and other biological fluids. Thanks to the use of highly avid and specific monoclonal antibodies, the format (microwell strips) and the short total assay time (6 hours) large numbers of samples can be readily handled in an economic way.
This kit will allow the quantitation of all four human IgG subclasses in 17 test samples.

2 PRINCIPLE OF THE TEST

The human IgG subclass ELISA combi kit is based on the sandwich ELISA principle. Microwell strips are coated with monoclonal antibodies, specific for one of the human IgG subclasses. Standard, control and test samples are incubated in the wells and the IgG subclass antigen is bound to the solid phase. Unbound material is removed by washing. Next, rabbit anti-human IgG antibody labelled to horse radish peroxidase (conjugate) is added to each well and unbound conjugate is removed by washing.
Conjugate binding is visualized by incubation with 2,2 azino-bis 3-ethylbenzthia-zoline-6-sulfonic acid di-ammonium salt (ABTS) and hydrogen peroxidase. The reaction is stopped with an acid buffer.
The green colour developed is proportional to the amount of IgG subclass in the test sample.

3 STORAGE AND STABILITY

The kit should be stored upright at +4°C and can be used until the expiration date shown on the label.
For detailed storage and stability data after opening the contents of the kit we refer to section 7 (Assay procedure).

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4 CONTENTS OF THE KIT

Quantity	Kit component	Volume
2 plates	24x8 well strips; six for each subclass distinguished by a color code: IgG1=red; IgG2=black; IgG3=yellow; IgG4=green	
1 vial #	dilution buffer (10-fold concentrated)	60 ml
1 vial #	washing buffer (20-fold concentrated)	50 ml
1 vial #	substrate buffer (10-fold concentrated)	5 ml
1 vial #	ABTS stock solution	1 ml
1 vial #	hydrogen peroxidase stock solution	0.5 ml
1 vial #	stop buffer	20 ml
1 vial #	rabbit anti-human IgG HRP conjugate	0.1 ml
2 vials	standard serum	0.1 ml
2 vials	control serum	0.1 ml
6 pcs	adhesive seals	

contains thiomersal

sensitivity

Limmits M1551

The lower limmits for the subclass ELISA are as follows:

IgG1	**6**	**ng/ml**
IgG2	**23**	**ng/ml**
IgG3	**2.9**	**ng/ml**
IgG4	**3.7**	**ng/ml**

The upper limmits are not fixed since they depend on how many times the samples are diluted.

5 ADDITIONAL MATERIALS REQUIRED

- Distilled water for dilution of washing-, dilution- and substrate buffers.
- Pipetting devices for accurate delivery of volumes in the range of 10 to 5000 µl with disposable plastic tips.
- Eight-channel multipipette to deliver 50 and 100 µl.
- Glass tubes for making dilutions.
- Flasks, beakers, cilinders for preparation of reagents.
- An incubator (37°C).
- A standard ELISA washer or a 500 ml plastic squirt bottle for automatic or manual washing of the strips.
- A standard ELISA reader for measuring absorbance at 414 or 405 nm.

6 PRECAUTIONS FOR USE

Although all human materials in this kit have been found negative for HBsAg, anti-Hepatitis C and anti-HIV antibodies, all components of human origin should be considered as potentially infectious.

Reagents which contain thiomersal (marked with # section 4) may be toxic upon ingestion, inhalation or skin contact. The usual precautions in handling such reagents should be observed.

Only use the reagents and microtiter-strips supplied with the kit.

7 TEST SAMPLE COLLECTION AND HANDLING

Serum and other biological fluids (e.g. liquor cerebrospinalis) and culture supernatant may be tested. Allow bloodsamples collected by venepuncture to clot at 4°C up to 48 hours prior to use. Seperate serum from the clot. Fresh samples should be stored at 4°C and assayed within 24 hours. Samples to be stored for longer periods of time should be frozen undiluted at -20°C. Avoid repeated thawing and freezing. Do not use samples that are grossly hemolyzed, microbiologically contaminated or lipemic.

8 ASSAY PROCEDURE

All dilutions should be made in duplicate!
Bring all reagents to room temperature (18-20°C).

a MICROTITER PLATES

The kit contains 2 frames each consisting of twelve precoated strips of eight microwells, seperately sealed in a plastic pouch containing desiccant. The microwell strips are distinguished by a color code: IgG1=red; IgG2=black; IgG3=yellow and IgG4=green. After opening the plastic pouch remove the strips that are not required immediately. Repack these strips airtight in presence of desiccant in the plastic bag provided. Store at +4°C and use within one week.

b BUFFERS

Washing buffer
Calculate the quantity of washing buffer required (approximately 2 ml undiluted buffer per microwell strip) and prepare a working-strenght solution by diluting this 1:20 in distilled water before use. The working-strenght solution washing buffer can be stored up to one week at 2-8°C. Note: in the concentrated buffer salt crystals may appear when stored at 2-8°C. Before preparing the working-strenght buffer, first warm the concentrated buffer BRIEFLY to 37°C to dissolve the precipitate.

Dilution buffer
Calculate the quantity of dilution buffer required (approximately 2 ml undiluted buffer per microwell strip) and prepare a working-strenght solution by diluting the buffer 1:10 in distilled water before use.
The working-strenght solution dilution buffer can be stored up to one week at 2-8°C.

c STANDARD AND CONTROL SERA

Standard: Pipette 4990 µl of dilution buffer into a 10 ml tube and add 10 µl of standard serum. (initial dilution 1:500).
Pipette 1900 µl of dilution buffer in a 3 ml tube with 100 µl 1:500 diluted standard.
Make from this tube a serial (seven) 2-fold dilution series by always adding 1000 µl of the previous dilution to 1000 µl dilution buffer. Select for each IgG subclass the series of standard dilutions: IgG2, IgG3, and IgG4 1:10.000 - 1:160.000 and IgG1 1:80.000 - 1:1.280.000.

Standard serum
IgG subclass concentrations:

IgG1	7.1	g/l
IgG2	3.6	g/l
IgG3	0.46	g/l
IgG4	0.59	g/l

	dilution standard serum	vol. prev. dil. (µl)	vol. dil. buffer (µl)	IgG1 conc. ng/ml	IgG2 conc. ng/ml	IgG3 conc. ng/ml	IgG4 conc. ng/ml
	1:500	10	4990				
St1	1:10.000	100	1900		360	46	59
St2	1:20.000	1000	1000		180	23	30
St3	1:40.000	1000	1000		90	11.5	15
St4	1:80.000	1000	1000	89	45	5.8	7.4
St5	1:160.000	1000	1000	44	23	2.9	3.7
St6	1:320.000	1000	1000	22			
St7	1:640.000	1000	1000	11			
St8	1:1.280.000	1000	1000	6			

Control: Pipette 4990 µl of dilution buffer into a 10 ml tube and add 10 µl of reconstituted control serum. (initial dilution 1:500).
Pipette 885 µl of dilution buffer in a 3 ml tube with 15 µl 1:500 diluted control. This is the 1:30.000 dilution for the a-IgG2, a-IgG3 and a-IgG4 test.
Prepare from this dilution the 1:240.000 dilution for the a-IgG1 test by mixing 875 µl of dilution buffer with 125 µl of the 1:30.000 dilution.

Prepare 1 tube of 3 ml with 1 ml dilution buffer as blank.

dilution control serum	vol. prev. dil. (µl)	vol. dil. buffer (µl)
undiluted		
1:500	10	4990
1:30.000	15	885
1:240.000	125	875

Test samples: Dilute test samples with dilution buffer according to the same protocol as for the control serum.

In case of myeloma proteins and pediatric or partly deficient sera, use dilutions respectively higher and lower in order to bring the IgG subclass concentration into the test range of the ELISA.

d **FIRST WASH STEP**
Wash the required microwell strips four times with working-strenght washing buffer in a microtiter plate washer. In case of manual washing, completely fill the wells with working-strenght washing buffer and aspirate, repeat this four times. After the final aspiration the wells should be dry.

e **FIRST INCUBATION STEP** standard, control and samples
Transfer 100 µl of the standard-, control- and sample dilutions and their duplicates into the appropriate wells. Transfer 100 µl of dilution buffer as a blank into two wells; a proposed plate-plan is shown on the last page.
Cover the wells with adhesive seal and incubate for 1 hour at 37°C.

f **SECOND WASH STEP**
Aspirate supernatant from wells and wash the microwell strips as discribed in point d.

g **SECOND INCUBATION STEP** anti-IgG conjugate
Dilute immediately prior to use the required amount of conjugate with dilution buffer.

dilution conjugate	vol. prev. dil. (µl)	vol. dil. buffer (µl)
undiluted		
1:250	40	9960
1:1250	3000	12.000

Add 100 µl of the 1:250 conjugate dilution to the a-IgG4 microwell strips and 100 µl of the 1:1250 conjugate dilution to the a-IgG1, a-IgG2 and a-IgG3
microwell strips using an eight-channel multipipette.
Cover the wells with a fresh adhesive seal and incubate for 1 hour at 37°C.

h **THIRD WASH STEP**
Aspirate supernatant from wells and wash the microwell strips as discribed in point d.

i **THIRD INCUBATION STEP** enzymatic colour development
During incubation of conjugate prepare substrate solution (approximately 900 µl per microwell strip will be needed).
Add the equivalents of 200 µl of hydrogen peroxide stock solution and 400 of ABTS stock solution to 20 ml of working-strenght substrate dilution (2 ml substrate stock solution + 18 ml distilled water).
Add 100 µl of substrate solution to all wells and incubate for 30 minutes at room temperature (18-22°C).
Cover the strips with adhesive seal.

j **STOP ENZYMATIC REACTION**
Add 50 µl of "ready to use" stop buffer to all wells.

k **PLATE READ-OUT**
Read at 414 (preferably) or 405 nm in an ELISA reader.
After stopping, the colour is stable for at least 8 hours.

9 **CALCULATIONS**

- Record the absorbance at 414 nm for each well and average the duplicates. For each sample duplicates should not differ more than 15% from the mean. If duplicates vary more the assay should be repeated. Test samples that show a mean absorbancy outside the range of the standard curve dilutions should be diluted appropriately or applied neat.

- On semi log paper plot the mean absorbancies of the standard curve on the ordinate (linear scale) versus the corresponding IgG subclass concentration in ng/ml on the abscissa (log scale).
For IgG subclass concentrations of the standard curve dilutions we refer to point 7c.
Draw or calculate a line of best fit through the five points.
Read or calculate the relative IgG subclass level in the control and test sample dilutions by interpolation of the standard curve.
Transform the relative IgG subclass level to the actual level by multiplication with the dilution factor of the test sample (see point 7c).

The IgG subclass levels of the control serum should be within the values given below:

IgG subclass	concentration (g/l)	range (g/l)
IgG1	4.12	3.05-5.16
IgG2	2.51	1.85-3.15
IgG3	0.31	0.23-0.39
IgG4	0.38	0.28-0.48

If the IgG subclass levels of the control serum are outside the specified range, the assay should be repeated.

To evaluate the test results, the IgG subclass levels in test samples should be compared to the normal values in children and adults as indicated below.

NORMAL IgG SUBCLASS LEVELS (g/l)

Age	IgG1	IgG2	IgG3	IgG4
0 - 1 month	2.4 - 10.6	0.87 - 4.1	0.14 - 0.55	0.04 - 0.56
1 - 4 mnths	1.8 - 6.7	0.38 - 2.1	0.14 - 0.70	<0.03 - 0.36
4 - 6 mnths	1.8 - 7.0	0.34 - 2.1	0.15 - 0.80	<0.03 - 0.23
6 - 12 mnths	2.0 - 7.7	0.34 - 2.3	0.15 - 0.97	<0.03 - 0.43
1 - 1.5 yrs	2.5 - 8.2	0.38 - 2.4	0.15 - 1.07	<0.03 - 0.62
1.5 - 2 yrs	2.9 - 8.5	0.45 - 2.6	0.15 - 1.13	<0.03 - 0.79
2 - 3 yrs	3.2 - 9.0	0.52 - 2.8	0.14 - 1.20	<0.03 - 1.06
3 - 4 yrs	3.5 - 9.4	0.63 - 3.0	0.13 - 1.26	<0.03 - 1.27
4 - 6 yrs	3.7 - 10.0	0.72 - 3.4	0.13 - 1.33	<0.03 - 1.58
6 - 9 yrs	4.0 - 10.8	0.85 - 4.1	0.13 - 1.42	<0.03 - 1.89
9 - 12 yrs	4.0 - 11.5	0.98 - 4.8	0.15 - 1.49	0.03 - 2.10
12 - 18 yrs	3.7 - 12.8	1.06 - 6.1	0.18 - 1.63	0.04 - 2.30
above 18 yrs	4.9 - 11.4	1.50 - 6.4	0.20 - 1.10	0.08 - 1.40

10 SPECIFIC PERFORMANCE CHARACTERISTICS

a Reproducebility

To determine assay reproducebility a control serum was measured 18 times in seperate assays.
The following results were obtained:

	IgG1	IgG2	IgG3	IgG4
mean conc. g/l	4.3	2.3	0.32	0.40
coëff. of variation %	<15	<15	<15	<15

b Comparison ELISA kit versus conventional RID

The IgG subclass levels of 13 randomly selected serum samples determined by the conventional RID technique were compared with those found by the ELISA kit.
The following correlations were established:

Subclass	Number of samples	ELISA kit / RID mean value ratio	Correlation coëfficient
IgG1	15	0.89 ± 0.11	0.93
IgG2	16	1.13 ± 0.23	0.97
IgG3	14	0.94 ± 0.22	0.94
IgG4	13	1.00 ± 0.36	0.89

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11 REFERENCES

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2. A. Vlug and P. van Remortel. Structure and function of human IgG subclasses. Eur. Clin. Lab., 8, 26., 1989.

3. Protides of the biological Fluids 36, 1989. Ed. by M.D. Poulik. Pergamon Press.

PLATE PLAN

	IgG1	IgG1	IgG1	IgG1	IgG1	IgG1	IgG2	IgG2	IgG2	IgG2	IgG2	IgG2
A	St4	St4	3	3	10	10	St1	St1	3	3	10	10
B	St5	St5	4	4	11	11	St2	St2	4	4	11	11
C	St6	St6	5	5	12	12	St3	St3	5	5	12	12
D	St7	St7	6	6	13	13	St4	St4	6	6	13	13
E	St8	St8	7	7	14	14	St5	St5	7	7	14	14
F	1	1	8	8	15	15	1	1	8	8	15	15
G	2	2	9	9	16	16	2	2	9	9	16	16
H	Ctr	Ctr	Bl	Bl	17	17	Ctr	Ctr	Bl	Bl	17	17

	IgG3	IgG3	IgG3	IgG3	IgG3	IgG3	IgG4	IgG4	IgG4	IgG4	IgG4	IgG4
A	St1	St1	3	3	10	10	St1	St1	3	3	10	10
B	St2	St2	4	4	11	11	St2	St2	4	4	11	11
C	St3	St3	5	5	12	12	St3	St3	5	5	12	12
D	St4	St4	6	6	13	13	St4	St4	6	6	13	13
E	St5	St5	7	7	14	14	St5	St5	7	7	14	14
F	1	1	8	8	15	15	1	1	8	8	15	15
G	2	2	9	9	16	16	2	2	9	9	16	16
H	Ctr	Ctr	Bl	Bl	17	17	Ctr	Ctr	Bl	Bl	17	17

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