

PeliClass human IgG subclass ELISA kit Enzyme-linked immunosorbent assay

Catalog No: M1551

Size: six pre-coated 8-well strips for each of the four IgG subclasses

Test description

The PeliClass human subclass ELISA kit is a 'sandwich'-type enzyme immunoassay. The kit contains microwell strips coated with highly avid monoclonal antibodies, each specific for one of the human IgG subclasses. Test samples, calibrator- and control sera are incubated in the respective wells. The IgG subclass to be determined will bind to the solid phase and non-bound IgG is removed by washing. Next, peroxidase-conjugated anti-human IgG antiserum is added to each well and non-bound conjugate is removed by washing. After incubation with substrate solution (ABTS) and H₂O₂, the reaction is stopped with an acid buffer. The green colored reaction product is measured by absorbance and the concentration of IgG subclass in the test sample calculated relative to the values of a reference curve. IgG subclass control serum is assayed to check the validity of the calibration curves and the accuracy of the IgG subclass determinations. This assay is highly sensitive and specific and allows for accurate measurement of very low IgG subclass levels.

Kit contents

The kit contains six pre-coated 8-well strips for each of the four IgG subclasses and additional reagents.

- two pre-coated microtiter plates with six color-coded 8-well strips for each IgG subclass
- horseradish peroxidase-conjugated anti-human IgG antibodies
- calibration and control sera
- wash-, dilution-, substrate- and stop buffers
- ABTS and hydrogen peroxidase substrate stock solution
- reference values for human IgG subclass concentrations

Proposed plate plan

	IgG1						IgG					
	1	2	3	4	5	6	7	8	9	10	11	12
A	Cal4	Cal4	3	3	10	10	Cal1	Cal1	3	3	10	10
B	Cal5	Cal5	4	4	11	11	Cal2	Cal2	4	4	11	11
C	Cal6	Cal6	5	5	12	12	Cal3	Cal3	5	5	12	12
D	Cal7	Cal7	6	6	13	13	Cal4	Cal4	6	6	13	13
E	Cal8	Cal8	7	7	14	14	Cal5	Cal5	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						IgG4					
	1	2	3	4	5	6	7	8	9	10	11	12
A	Cal4	Cal4	3	3	10	10	Cal1	Cal1	3	3	10	10
B	Cal5	Cal5	4	4	11	11	Cal2	Cal2	4	4	11	11
C	Cal6	Cal6	5	5	12	12	Cal3	Cal3	5	5	12	12
D	Cal7	Cal7	6	6	13	13	Cal4	Cal4	6	6	13	13
E	Cal8	Cal8	7	7	14	14	Cal5	Cal5	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



ELISA kit for quantitative determination of human IgG subclasses in serum

I. INTRODUCTION

Human IgG comprises four subclasses: IgG1, IgG2, IgG3 and IgG4. The biochemical characteristics of the IgG subclasses have been described extensively. The differences between IgG subclasses are reflected in several biologically important functions such as antigen recognition, complement activation and binding to cell surface receptors. Many studies have revealed that abnormalities in serum levels of IgG subclasses may be associated with various disease states. Especially the association of selective IgG2 subclass deficiency with increased susceptibility to viral or bacterial infections has been amply documented. Low serum levels of IgG2 or IgG3 have been reported in patients with recurrent upper and lower respiratory tract infections. Others have found an association between very low IgG4 serum concentrations and recurrent sinopulmonary infections. Abnormalities in the serum levels of IgG subclasses have also been observed in autoimmune diseases, neurological disorders and in HIV infections.

II. TECHNICAL PRINCIPLE

The PeliClass human subclass ELISA kit is a 'sandwich'-type enzyme immunoassay. The kit contains microwell strips coated with highly avid monoclonal antibodies, each specific for one of the human IgG subclasses. Test samples, calibrator- and control sera are incubated in the respective wells. The IgG subclass to be determined will bind to the solid phase and non-bound IgG is removed by washing. Next, peroxidaseconjugated anti-human IgG antiserum is added to each well and non-bound conjugate is removed by washing. After incubation with substrate solution (ABTS) and H₂O₂, the reaction is stopped with an acid buffer. The green colored reaction product is measured by absorbance and the concentration of IgG subclass in the test sample calculated relative to the values of a reference curve. IgG subclass control serum is assayed to check the validity of the calibration curves and the accuracy of the IgG subclass determinations. The IgG subclass levels in the calibrator(s) were determined using a calibrator derived from the WHO 67/97 reference preparation. The recommended target values of 5.0 g/L for IgG1, 2.6 g/L for IgG2, 0.4 g/L for IgG3 and 0.5 g/L for IgG4 were used.

III. STORAGE AND STABILITY

The PeliClass human subclass ELISA kit should be stored upright at 2-8 °C. It can be used until the expiration date shown on the label. For all components stability after opening is 1 week when stored at 2-8 °C. Transport conditions may differ from storage conditions.

IV. CONTENTS OF THE KIT

The PeliClass human subclass ELISA kit contains sufficient reagents for 48 tests for each subclass, including calibrators, controls and blank. See the Table at the end of this package insert.

The monoclonal antibodies were purified from tissue culture medium, using column chromatography (ion exchange and affinity chromatography). The calibrator and control are liquid human sera.

V. ADDITIONAL MATERIALS REQUIRED

- Distilled water for dilution of wash- dilution- and substrate buffers.
- Pipetting devices for accurate delivery of volumes.
- An incubator (37 ± 2°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 nm or 405 nm.
- Log linear paper.

VI. TEST SAMPLE HANDLING

Only serum samples should be tested. The samples should be as fresh as possible or stored frozen. The samples should be manually diluted before use (see VII ASSAY PROTOCOL).

VII. ASSAY PROTOCOL

- Bring all reagents to room temperature (18-25°C) and mix thoroughly. Avoid bubbles or foam.
- It is advised to test all samples, controls and calibrator dilutions in duplicate.

1. MICROTITRE PLATE

The PeliClass human IgG subclass ELISA kit provides the flexibility to use partial plates on separate occasions. Before opening the plastic pouch, determine the number of strips required to test the desired number of samples plus 14 wells needed for running calibrators, controls and blanks in duplicate. Remove strips that will not be used from the plate-frame and re-pack them in the plastic pouch containing the desiccant and store at 2-8°C.

2. BUFFER PREPARATIONS

Wash buffer: Prepare the wash buffer by adding the total content of the bottle of the wash buffer concentrate to 950 mL distilled water. The diluted wash buffer must be stored at 2-8°C and remains stable for 1 week.

Note: The concentrated buffer may contain salt crystals. Before preparing the working-strength buffer, warm the concentrated buffer BRIEFLY to 37°C to dissolve the crystals.



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Dilution buffer: Calculate the quantity of dilution buffer required (approximately 2 mL undiluted buffer per microwell strip) and prepare a working-strength solution by diluting the buffer 10 fold in distilled water.

3. PREPARATION OF THE CALIBRATOR AND CONTROL SERA

For concentrations see Table 2 and 3 of the enclosed information leaflet.

Calibrator: (See table 1 of the enclosed information leaflet) Label one 10 mL tube with '1:500' and eight 3 mL tubes with 'Cal1 to Cal8' respectively.

Pipette 4.99 mL of dilution buffer into the 10 mL tube and add 10 µL of calibrator serum (initial dilution 1:500). Pipette 1.9 mL of dilution buffer in the tube labeled 'Cal1' and 1.0 mL into the tubes labeled 'Cal2 - Cal8'.

Pipette 100 µL 1:500 diluted calibrator to the tube Cal1, and make from this tube seven 2-fold serial dilutions by adding 1.0 mL from the previous dilution to the next 'Cal' tube. Select for each IgG subclass the series of calibrator dilutions: IgG1 1:80,000 -1:1,280,000 IgG2, IgG3 and IgG4 1:10,000 -1:160,000.

Control: Label one tube with '1:500', and two 3 mL tubes with '1:30,000' (for IgG2, 3, 4) and '1:240,000' (for IgG1) respectively.

Pipette in the tube labeled with 1:500 @ 4.99 mL dilution buffer and 10 µL control serum.

Pipette in the tube labeled with 1:30,000 @ 885 µL dilution buffer and 15 µL 1:500 dilution.

Pipette in the tube labeled with 1:240,000 @ 875 µL dilution buffer and 125 µL of the 1:30,000 dilution.

Prepare one tube of 3 mL with 1 mL dilution buffer as blank.

4. PREPARATION OF SAMPLES

Dilute the test samples with dilution buffer according to the same protocol as for the control serum. For results that lie outside the given ranges (see Table 1 of the enclosed information leaflet), the test should be repeated with a different sample dilution.

5. FIRST WASH STEP

Wash the required microwells in the plate-frame four times with washing buffer. For manual washing, completely fill the wells (> 300 µL) with washing buffer and discard, repeat this procedure three times. Finally, the wells should be completely empty. Subsequent reagent should be added immediately, do not let the wells stand dry for extended period of time.

6. FIRST INCUBATION STEP

Add 100 µL of the diluted calibrators, controls, samples and blanks into the appropriate wells.

Cover plate with adhesive seal, gently agitate by tapping the edge of the microtitreplate for a few seconds to mix contents of each well.

Incubate for 1 hour at 37°C.

Just before washing, prepare next incubation reagent as described in point 8.

7. SECOND WASH STEP

Aspirate supernatant from the wells and wash the plate as described in point 5.

8. INCUBATION WITH HRP-CONJUGATED ANTIBODY TO HUMAN IgG

Dilute the conjugate 1:500 by pipetting 30 µL of the conjugate to 14.97 mL dilution buffer. Dilute the conjugate further to:

1:3000 by pipetting 1.2 mL of the 1:500 dilution to 6.0 mL dilution buffer.

1:2000 by pipetting 2.0 mL of the 1:500 dilution to 6.0 mL dilution buffer.

1:1000 by pipetting 3.5 mL of the 1:500 dilution to 3.5 mL dilution buffer.

Add: 100 µL of the 1:500 dilution to the anti-IgG1 microwell strips;

100 µL of the 1:3000 dilution to the anti-IgG2 microwell strips;

100 µL of the 1:2000 dilution to the anti-IgG3 microwell strips;

100 µL of the 1:1000 dilution to the anti-IgG4 microwell strips.

Cover the plate with adhesive seal, gently agitate by tapping the edge for a few seconds to mix the contents of each well.

Incubate for 1 hour at 37°C.

Just before washing prepare next incubation reagent as described in point 10.

9. THIRD WASH STEP

Aspirate supernatant from the wells and wash the plate as described in point 5.

10. INCUBATION WITH ABTS-SUBSTRATE

Calculate the amount of substrate solution (approximately 0.9 mL per microwell strip will be needed).

Add the equivalents of 200 µL hydrogen peroxide stock solution and 400 µL ABTS stock solution to 20 mL of working strength substrate buffer (2 mL substrate stock solution + 18 mL distilled water).

Add 100 µL of substrate solution to all wells.

Gently agitate by tapping the edge of the microtitreplate for a few seconds to mix the contents of each well.

Incubate for 30 minutes at room temperature (18-25°C).

11. STOP ENZYMATIC REACTION



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Add 50 µL of stop solution to all wells.

12. PLATE READ-OUT

Read **within 1 hour** at 414 (preferably) or 405 nm in an ELISA reader.

VIII. RESULTS AND INTERPRETATION OF DATA

- Record the absorbance at 414 (preferably) or 405 nm for each well and average the duplicate values.
- For each sample, duplicates should not differ more than 15% from the mean value. If duplicates vary more, the assay should be repeated.
- Plot the average absorbances of the calibrators (y-axis) versus the related subclass concentration in ng/mL (x-axis) on log-linear paper and draw the best fitting curve.
- The IgG subclass levels of the control serum should lie within the range(s) given in Table 3 of the enclosed information leaflet.
- Interpolate the average absorbances value for each sample on the reference curve.
- Test samples which show a mean absorbance outside the range of the reference curve dilutions, should be diluted appropriately.
- For an evaluation of the IgG subclass concentration in a test sample, compare the levels found with the normal values for IgG subclasses (see X REFERENCE RANGES).

IX. ASSAY RANGES

Consult Table 1 of the enclosed information leaflet for the kit specific assay ranges.

X. REFERENCE RANGES

Reference ranges (g/L) for IgG subclasses in serum samples of healthy Caucasian individuals. For other populations separate reference ranges should be obtained.

Age				IgG1	IgG2	IgG3	IgG4
0	-	1	mnth	2.4 - 10.6	0.87 - 4.1	0.14 - 0.55	0.04 - 0.56
1	-	4	mnth	1.8 - 6.7	0.38 - 2.1	0.14 - 0.70	<0.03 - 0.36
4	-	6	mnth	1.8 - 7.0	0.34 - 2.1	0.15 - 0.80	<0.03 - 0.23
6	-	12	mnth	2.0 - 7.7	0.34 - 2.3	0.15 - 0.97	<0.03 - 0.43
1	-	1½	yr	2.5 - 8.2	0.38 - 2.4	0.15 - 1.07	<0.03 - 0.62
1½	-	2	yr	2.9 - 8.5	0.45 - 2.6	0.15 - 1.13	<0.03 - 0.79
2	-	3	yr	3.2 - 9.0	0.52 - 2.8	0.14 - 1.20	<0.03 - 1.06
3	-	4	yr	3.5 - 9.4	0.63 - 3.0	0.13 - 1.26	<0.03 - 1.27
4	-	6	yr	3.7 - 10.0	0.72 - 3.4	0.13 - 1.33	<0.03 - 1.58
6	-	9	yr	4.0 - 10.8	0.85 - 4.1	0.13 - 1.42	<0.03 - 1.89
9	-	12	yr	4.0 - 11.5	0.98 - 4.8	0.15 - 1.49	0.03 - 2.10
12	-	18	yr	3.7 - 12.8	1.06 - 6.1	0.18 - 1.63	0.04 - 2.30
	>	18	yr	4.9 - 11.4	1.50 - 6.4	0.20 - 1.10	0.08 - 1.40

XI. SPECIFIC PERFORMANCE CHARACTERISTICS

a Reproducibility

IgG subclass concentration	IgG1	IgG2	IgG3	IgG4
Intra-assay variation (%)	4	3	4	2
Inter-assay variation (%)	2	2	7	7

b Comparison of PeliClass human IgG subclass kit vs. Mancini.

The IgG1, IgG2, IgG3 and IgG4 concentrations in sera were determined by an ELISA assay and compared with the corresponding values found in Mancini. The following correlations were established:



IgG subclass	linear regression line	correlation
IgG1	$Y = 0.90X - 0.29$	0.97
IgG2	$Y = 1.01X - 0.17$	0.93
IgG3	$Y = 0.91X + 0.00$	0.99
IgG4	$Y = 0.96X - 0.03$	0.98

Note:

The values cited for specific performance characteristics of the test represent typical results and are not to be viewed as specifications for this kit.

XII. RESTRICTIONS

1. User should be trained and familiar with ELISA assays and test procedure.
2. Grossly hemolyzed or lipemic samples should not be used. Unexpected results may be obtained for samples containing rheumatoid factor, high bilirubin levels, or other circulating immune complexes. These samples should be analyzed by other method.
3. Out of range samples, e.g. in case of paraproteins, should be repeated using different dilutions.
4. The finding of a decreased level of one of the IgG subclasses can never provide a definite diagnosis, but should rather be considered as an indication of a disturbance of the immune system, requiring further diagnostic investigation.
5. Control serum should always be used to check the validity of the calibration curves. When the control is out of range, the results of the test samples are not reliable. The test should be repeated.
6. Reagents from different batches are not interchangeable.
7. Rests of reagents (e.g. dead volume) should not be mixed with contents of freshly opened vials.
8. Caps and vials are not interchangeable. Caps should be replaced on the corresponding vials.
9. Although the human calibrator and control sera have been tested for the markers of specific disease transmitting agents in accordance with current EU guidelines to GMP and found to be nonreactive, all components of human origin should be considered as potentially infectious.
10. Preservative: Thiomersal® 0,001%.
11. Use new plate seals for each incubation/fixation step in the ELISA-experiment to avoid cross contamination. Do not use aluminum foil.
12. Use disposable pipette tips for each transfer to avoid cross contamination.
13. Each time the kit is used, fresh dilutions of calibrators, conjugate and buffers should be made.
14. Do not use other reagents and microtiter strips than those supplied with the kit.
15. Sodium azide inactivates HRP, do not use sodium azide-containing solutions, nor add sodium azide to the supplied buffers.
16. The waste-disposal should be performed according to your laboratory regulations.

Not for human use. For research only. Not for use in diagnostic or therapeutic procedures.



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