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Mouse-IgG ELISA

Version 11

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Enzyme immunoassay for the determination of mouse-IgG in mouse hybridoma supernatants and ascites.

Cat. No. 11 333 151 001

Kit for 400 tests

Store the kit at +2 to +8°C

Table of Contents

1. What this Product Does

Kit Contents

Vial/Cap	Label	Content
1 red	Capture antibody	1 vial anti-mouse-Fcγ from sheep, immunosorptively purified, lyophilized.
2 red	Coating buffer concentrate	Sodium carbonate buffer, 0.5 M sodium azide, 0.95% (w/v); pH 9.4 – 9.7.
3 white	Detergent	One vial with Tween 20.
4 green	Blocking reagent	Powder mixture consisting of a peptide mixture obtained by proteolysis of gelatin; Tris-HCl buffer and NaCl.
5 yellow	Standard	One vial with monoclonal mouse-IgG2a, immunosorptively purified, lyophilized
6 purple	Conjugate	One vial with conjugate mixture (anti-mouse-κ-POD and anti-mouse-λ-POD) from sheep, Fab-fragments, immunosorptively purified, lyophilized.
7 green	Substrate buffer	One bottle with buffer mixture consisting of sodium perborate and citric acid/sodium phosphate buffer.
8 white	ABTS substrate tablets	One vial with ABTS substrate tablets (5 mg ABTS per tablet).
	20 empty vials	(1.5 ml) with screw caps and rubber seals (airtight closeable) in a polystyrene rack for the aliquotation of coating antibody and standard solutions.

Product Overview The Mouse IgG ELISA follows a standard sandwich ELISA protocol.

❶	A special catching antibody is bound adsorptively to the wells of microplates.
❷	After blocking with Blocking reagent the antibody contained in the sample (*e.g.,* hybridoma-supernatant, ascites dilution, etc.) is bound to the capture antibody during a further incubation step.
❸	POD is fixed to the monoclonal antibody using a POD-labeled, balanced mixture of anti-mouse-κ- and anti-mouse-λ-antibodies (immunosorbed Fab-fragments).
❹	With the highly sensitive ABTS-perborate system a dark green color is formed in the reaction with the fixed peroxidases. Evaluation is performed using a standard graph.

Coating Antibody The coating antibody is a immunosorptively purified, polyclonal anti-mouse-Fcγ from sheep with a particularly balanced composition to allow recognition of all IgG-subclasses (including IgG3).

Application The kit can be used for the determination of mouse-IgG in hybridoma-supernatants, ascites, mouse-sera, etc.

Specificity The mouse-IgG ELISA shows no cross-reactivity with other Ig-classes from mouse or bovine-IgG (<0.1 %).

Signals of all IgG-subclasses from mouse (IgG1, IgG2a, IgG2b and IgG3) can be detected independently of the type of light chains (κ or λ).

In contrast, other Ig-subclasses (IgM, IgA, etc.) will not be detected with this assay.

Sensitivity In the procedure described the assay shows a detection limit of approx. 10 ng IgG/ml.

If necessary the sensitivity can be increased by the following methods: doubling the capture antibody-concentration; doubling the conjugate-concentration; doubling the incubation period with capture antibody and with conjugate.

Storage/Stability The kit is stable at +2 to +8°C until the expiration date printed on the label.

2. How to Use this Product

2.1 Before you Begin

Antibody Fragments

Due to the nature of the test principle no signal will be obtained *e.g.,* with Fcγ- and Fab-fragments or free heavy- or light-chains.

"Stressed" (Partly Denaturated) Antibodies

To elute antibodies from affinity columns (immobilized antigens) or from protein A- or protein G-agarose, rather stringent denaturation conditions (*e.g.,* glycine-HCl; pH 2.8; propionic acid, 1 M etc.) are necessary in some cases.

Since the mouse IgG-ELISA is very sensitive to this kind of denaturation, quantitative determination of antibodies treated by such conditions is therefore not recommended. In contrast buffers (Na- or K-phosphate, Tris-HCl, NaCl, etc.) used during standard chromatography techniques (*e.g.,*, ion exchange chromatography; gel permeation chromatography; etc.) do not influence the assay.

Pipetting

Care must be taken to ensure that the volume of the antibody and the conjugate solution applied is not in any case larger than the volume of the Blocking reagent in order to avoid contact with uncoated surfaces.

Additional Reagents and Equipment Required

- Sodium chloride (NaCl), A. R.
- Thymol crystals
- Microplates "MicroWell" microplates (with flat bottoms) from Nunc GmbH, Wiesbaden, Germany, have been used for this assay.

Preparation of Working Solutions

Please refer to the following table:

Component	Preparation	Storage/stability
Coating buffer	Dilute 10 ml Coating buffer-concentrate (bottle 2) with double dist. water to 100 ml (1:10). ⚠ Seal hermetically to avoid contact with CO_2	at +2 to +8°C
Capture antibody	Dissolve Capture antibody lyophilizate (bottle 1) in 0.5 ml double dist. water. Pipette 10 aliquots (50 µl each) into 10 of the enclosed empty vials (9)	For several months when stored in aliquots at −15 to −25°C; avoid more than 3 thaw/freeze cycles.
Capture antibody solution	25 µl Capture antibody is used together with 1 ml Coating buffer. ⚠ Sufficient for 20 determinations.	Prepare shortly before use!

Component	Preparation	Storage/stability
Wash solution	NaCl, 0.9% (w/v); Tween 20, 0.1% (v/v)] Dissolve 1 ml Detergent (Tween 20, vial 3) and 9 g NaCl in double dist. water and fill up to 1000 ml. ⚠ Warm Detergent (vial 3) up to +15 to +25°C before use.	at +2 to +8°C.
Blocking reagent concentrate	Dissolve the contents of one bottle Blocking reagent (bottle 4) in 100 ml double dist. water while stirring (for approx. 30 min).	Store in aliquots at −15 to −25°C.
Blocking reagent 1×	Dilute 25 ml Blocking reagent concentrate with double dist. water to 250 ml (1:10).	Store in aliquots at −15 to −25°C.
Conjugate solution	Dissolve the conjugate (bottle 6) in 1.0 ml double dist. water and add some thymol crystals until saturation to prevent microbial contamination.	Stable for at least 6 months when stored at +2 to +8°C. Do not freeze!
Conjugate dilution	Dilute 50 µl conjugate solution with Blocking reagent to 1 ml. ⚠ Sufficient for 20 determinations.	Prepare conjugate dilution only shortly before use.
Substrate solution	Dissolve 1 ABTS substrate tablet (bottle 8) in 5 ml Substrate buffer (bottle 7).	Approx. 3 months when stored protected from light at +2 to +8°C.

Preparation of Standard Solution

- Open standard (bottle 5) very carefully avoiding any loss of lyophilizate material and add exactly 500 µl double dist. water.

⚠ On the label of vial 5 the contents of IgG per vial is given in mg/fl (flask). Since the contents of the vial have to be dissolved in 0.5 ml, the IgG concentration (in mg/ml) is exactly twice the figure printed on the label after reconstitution.

- Close bottle carefully, allow to stand at +15 to +25°C for 30 min and then dissolve completely the contents of the bottle by gentle swirling.

⚠ Avoid the formation of foam and do not shake.

After complete reconstitution pipette 10 aliquots (50 µl each) into the remaining 10 empty vials (9), close tightly. Stable for several months when stored in aliquots at −15 to −25°C; avoid more than 3 thaw/freeze cycles.

Preparation of Standard Dilutions

The reconstituted Standard solution contains approx. 0.1 mg IgG/vial (approx. 0.2 mg IgG/ml). Exact, lot-specific information about the concentration is given on the label (bottle 5).

⚠ On the label of vial 5 the contents of IgG per vial is given in mg/flask. Since the contents of the vial have to be dissolved in 0.5 ml, the IgG concentration (in mg/ml) is exactly twice the figure printed on the label after reconstitution.

- Pipette the following dilution series: 200, 100, 25, 12.5, 6.25 ng/ml.
- Prepare standard dilutions only shortly before use.
- Double estimations are recommended.

Tube	Dilution	From Tube..	Dilution Buffer (1× Blocking reagent)	Final Conc.
A	1:200	20 µl from vial 5*	3980 µl	1000 ng/ml
B	1:5	200 µl from A	800 µl	200 ng/ml
C	1:2	500 µl from B	500 µl	100 ng/ml
D	1:2	500 µl from C	500 µl	50 ng/ml
E	1:2	500 µl from D	500 µl	25 ng/ml
F	1:2	500 µl from E	500 µl	12.5 ng/ml
G	1:2	500 µl from F	500 µl	6.25 ng/ml

*please see label for exact concentration

Preparation of Sample

- Dilute hybridoma-supernatants and ascites according to the titer with Blocking reagent or culture medium until the level of the standard graph (25 - 80 ng IgG/ml) is reached (approx. 1:100 to 1:1000 for hybridoma-supernatants and approx. 1:10,000 to 1:100,000 for ascites).
- Double estimations are recommended.

2.2 Assay Procedure

Preparation of Sample

Evaluation is carried out with the aid of a microplate reader at a wavelength of $\lambda = 405$ nm. The standard graph will be plotted onto semi-logarithmic paper (extinction versus log concentration) and the concentration of the samples can be read from the standard graph (modern microplate readers use computer programs to print out directly the analyte concentrations determined mathematically by the standard graph). In contrast to immunoglobulins with light-chains of the κ-type, IgG-molecules with λ light-chains, which are relatively rare (approx. 3% of the whole population), show a somewhat narrower "linear" range, and the absorbency tends to level off at lower concentrations with this assay. In this case only concentrations <80 ng/ml should be read from the standard graph.

Protocol

Please refer to the following table. Incubations can be performed with (700 rpm, *e.g.,* IKA shaker MTS) or without shaking at +15 to +25°C.

⚠ Handling time for the assay without shaking will be approx. 4.5 and with shaking approx. 2.5 h

❶	Pipette 50 µl Capture antibody solution into each well and incubate.	
	Without shaking	**With shaking**
	1h	30 min
❷	Remove the solutions from the wells first by pouring out, then by tapping on a clean dry cloth (*e.g.,*, cellulose).	
❸	Wash with 200 µl Wash solution per well. Allow to take effect for 15 s, pour out and tap. Repeat washing twice.	
❹	Pipette 200 µl Blocking reagent (1×) into each well and incubate	
	Without shaking	**With shaking**
	15 min	15 min
❺	Remove the solutions from the wells first by pouring out, then by tapping on a clean dry cloth (*e.g.,* cellulose).	
❻	Wash with 200 µl Wash solution per well. Allow to take effect for 15 s, pour out and tap. Repeat washing twice.	
❼	Pipette 50 µl Antibody solution into each well and incubate	
	Without shaking	**With shaking**
	1h	30 min
❽	Remove the solutions from the wells first by pouring out, then by tapping on a clean dry cloth (*e.g.,* cellulose).	
❾	Wash with 200 µl Wash solution per well. Allow to take effect for 15 s, pour out and tap. Repeat washing twice.	
❿	Pipette 50 µl Conjugate dilution into each well and incubate	
	Without shaking	**With shaking**
	1h	30 min
⓫	Remove the solutions from the wells first by pouring out, then by tapping on a clean dry cloth (*e.g.,* cellulose).	
⓬	Wash with 200 µl Wash solution per well. Allow to take effect for 15 s, pour out and tap. Repeat washing twice.	
⓭	Pipette 50 µl Substrate solution into each well and incubate	
	Without shaking	With shaking
	1h	30 min

3. Troubleshooting

Please refer to the following table.

⚠ A further reason for a high background and/or too weak color development may be the quality of the microplates. Possibly change the lot or the supplier.

	Possible cause	Recommendation
No or too weak color development.	Capture antibody is not active (*e.g.,*, due to repeated thawing and freezing).	• Use a fresh (not yet thawed) aliquot of Capture antibody. • Increase the concentration of the Capture antibody or of the Conjugate solution, respectively (see "Sensitivity").
	Standard graph is too shallow.	Use a fresh (not yet thawed) aliquot (standard).
	Values of an antibody sample to be measured is obviously too low	• The antibody has been treated with denaturing agents (see "stressed" antibodies). Also commercially available antibodies may have been produced under denaturing conditions.
	Conjugate solution or Conjugate dilution is inactivated by incorrect storage or storage for too long periods.	• Prepare fresh Conjugate solution or Conjugate dilution. • Store at +2 to +8°C, do not freeze. • Increase the concentration of the Capture antibody or of the Conjugate solution, respectively (see “Sensitivity”).
	Substrate solution has been incorrectly stored or is too old.	• Prepare fresh Substrate solution. • Store in dark at +2 to +8°C.
High background	Substrate solution is too old (color changes from light green to dark green).	• Prepare fresh Substrate solution. • $E_{405\ nm}$/1 cm should be <0.16.
	Inadequate blocking.	Extend Blocking step to 30 min.
	The monoclonal antibodies (*e.g.,* anti- collagen) bind to the peptides in the Blocking reagent.	Replace Blocking reagent by: Tris-HCl, 50 mM; NaCl, 150 mM; pH 7.5; bovine serum albumin, 1% (w/v)

4. Supplementary Information

4.1 Conventions

4.1.1 Text Conventions

To make information consistent and memorable, the following text conventions are used in this document:

Text Convention	Usage
Numbered instructions labeled ❶, ❷, etc.	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Diagnostics.

4.1.2 Symbols

In this document, the following symbols are used to highlight important information:

Symbol	Description
⚠	Important Note: Information critical to the success of the procedure or use of the product.

4.2 Changes to Previous Version

- Editorial changes.

4.3 Ordering Information

Product	Pack size	Cat. No.
BM-Cyclin	37.5 mg (for 2× 2.5 l medium)	10 799 050 001
Geneticin (G 418)	1 g 5 g, non-sterile	11 464 981 001 11 464 990 001
Gentamicin (Gentamicin sulfate)	20 ml (500×), sterile	11 059 467 001
Hygromycin B from Streptomyces hygroscopicus	1 g (20 ml), sterile	10 843 555 001
Kanamycin (Kanamycin sulfate)	20 ml (500×), sterile	11 074 466 001

Product	Pack size	Cat. No.
Penicillin- Streptomycin (500×) for cell culture sterile	for 20 ml (500×) lyophilizate, sterile	11 074 440 001
Insulin-Transferrin-Sodium Selenite Supplement	50 mg (for 5 l medium)	11 074 547 001
Transferrin from human serum	20 ml (30 mg/ml; in IMDM, sterile)	10 652 202 001
Nutridoma-CS	10 ml, sterile	11 363 743 001
Nutridoma-SP	100 ml, sterile	11 011 375 001
BM-Condimed	100 ml, sterile	10 663 573 001
Isostrip Mouse Monoclonal Antibody Isotoping Kit	1 kit (10 tests)	11 493 027 001
BM Condimed H1 (Hybridoma Clonong Supplement)	100 ml (10×)	11 088 947 001
Hybridoma Fusion and Cloning supplement (HFCS)	10 ml (50×)	11 363 735 001
Polyethylen Glycol 1500 (PEG 1500)	10 × 4 ml	10 783 641 001

4.4 Trademarks

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4.5 Regulatory Disclaimer

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