RAT MONOCLONAL ANTIBODIES ANTI-MOUSE IMMUNOGLOBULINS

Supplied by: dianova GmbH

Warburgstrasse 45 ● 20354 Hamburg

Phone: +49 (0)40 45 06 70 ● Email: info@dianova.de www.dianova.com

dianova GmbH ● Warburgstrasse 45 ● 20354 Hamburg, Germany

Phone: +49 (0)40 45 06 70 ● Email: info@dianova.de● www.dianova.com

MOUSE IMMUNOGLOBULINS[1]

(Sub)classes	IgM	IgD	IgG1	IgG2a	IgG2b	IgG3	IgE	IgA
Heavy chains	mu	delta	gamma1	gamma2a	gamma2b	gamma3	epsilon	alpha
Light chains	Approximately 95% of kappa - 5% of lambda							
Molecular weight (KD)	900	180	160				190	170-500
Serum concentrations (per ml)	0.1-1.0 mg	1-10 µg	1-3 mg	1-10 mg	1-5 mg	0.1-0.2 mg	0.1-1 µg	1-3 mg
Serum half-life (days)	0.5	-	8-11	6-12	3-3.5	4	0.5	0.5

[1] References: Bazin and Malet, Immunology 1969, 17: 345; Bazin and Duplan, Rev. Fr. & Clin. Biol. 1966, 11: 987; Ey et al., Immunochemistry 1978, 15: 429; Fahey et al., J. Exp. Med. 1964, 120: 223; Fahey and Sell, J. Exp. Med. 1965, 122: 41; Grey et al., J. Exp. Med. 1971, 133: 289; Hirano et al., Int. Arch. Allergy 1983, 71: 182; Potter, Phys. Rev. 1972, 52: 631.

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ANTI-LIGHT CHAIN

MAb	Species of the Mab and isotype	Specificity mouse immunoglobulin	Avidity* tested on	Avidity* (M^{-1})	Recommended applications Immunoassay**	Recommended applications Immunopurification
LO-MK-1	rat IgG2a kappa	kappa light chain	IgM IgG1 IgG2a IgG2b IgG3	ND ND $3x10^9$ ND ND	yes	No (all isotypes)
LO-MK-2	rat IgG1 kappa	kappa light chain	IgM IgG1 IgG2a IgG2b IgG3	$2x10^8$ $1x10^9$ $3x10^9$ $2x10^9$ $2x10^9$	yes	yes (all isotypes)

*See technical data sheet for more details. **See technical data sheet for labelling properties. ND: not determined.

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ANTI-HEAVY CHAIN

MAb	Species of the MAb and isotype	Specificity mouse immunoglobulin	Avidity* (M^{-1})	Recommended applications Immunoassay**	Immunopurification
LO-MM-3	rat IgM kappa	mu heavy chain	2×10^{8}	yes	yes
LO-MM-8	rat IgG1 kappa	mu heavy chain	3.6×10^{9}	yes	ND
LO-MM-9	rat IgG2a kappa	mu heavy chain	7×10^{8}	yes	yes
LO-MA-7	rat IgM kappa	alpha heavy chain	4.8×10^{9}	yes	yes
LO-MA-10	rat IgM kappa	alpha heavy chain	2.9×10^{9}	yes	no
LO-MD-6	rat IgG2a kappa	delta heavy chain	1.2×10^{9}	yes	yes
LO-MD-8	rat IgG1 kappa	delta heavy chain	2.9×10^{9}	yes	ND
LO-ME-2	rat IgG2a kappa	epsilon heavy chain	2×10^{9}	yes	yes
LO-ME-3	rat IgG1 kappa	epsilon heavy chain	3×10^{9}	yes	ND
LO-MG1-2	rat IgG1 kappa	gamma 1 heavy chain	9×10^{8}	yes	yes
LO-MG1-13	rat IgG1 kappa	gamma 1 heavy chain	5.1×10^{9}	yes	ND
LO-MG1-15	rat IgG1 kappa	gamma 1 heavy chain	2.8×10^{9}	yes	ND
LO-MG2a-2	rat IgG2a kappa	gamma 2a heavy chain	7×10^{9}	yes	yes
LO-MG2a-3	rat IgG2a kappa	gamma 2a heavy chain	3×10^{9}	yes	yes
LO-MG2a-7	rat IgG1 kappa	gamma 2a heavy chain	5.1×10^{9}	yes	ND
LO-MG2a-9	rat IgG1 kappa	gamma 2a heavy chain	2.5×10^{9}	yes	ND
LO-MG2b-1	rat IgG1 lambda	gamma 2b heavy chain	3×10^{8}	yes	yes
LO-MG2b-2	rat IgG1 kappa	gamma 2b heavy chain	1×10^{10}	yes	yes
LO-MG3-7	rat IgM kappa	gamma 3 heavy chain	2×10^{10}	yes	no
LO-MG3-13	rat IgG1 kappa	gamma 3 heavy chain	6.8×10^{9}	yes	yes

*See technical data sheet for more details. **See technical data sheet for labelling properties. ND: not determined

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AVIDITY (1) OF RAT MONOCLONAL ANTIBODIES ANTI-MOUSE IMMUNOGLOBULIN (M^{-1})

Tested on isotypes proteins	IgM PB1 (2)	IgA ABPC-105	IgG1 polyclonal (3)	IgG2a MOPC-173(4)	IgG2b 307-E9 (2)	IgG3 C8302C11 (2)	IgE LB-4 (2,5)	IgD TEPC1017 TEPC1033 (6)
LO-MK-1 LO-MK-2	2×10^8		1×10^9	3×10^9 3×10^9	2×10^9	2×10^9		
LO-MM-3 LO-MM-8 LO-MM-9	2×10^8 3.6×10^9 2×10^8							
LO-MA-7 LO-MA-10		4.8×10^9 2.9×10^9						
LO-MD-6 LO-MD-8								1.2×10^9 2.9×10^9
LO-ME-2 LO-ME-3							2×10^9 3×10^9	
LO-MG1-2 LO-MG1-13 LO-MG1-15			9×10^8 5.1×10^9 2.8×10^9					
LO-MG2a-2 LO-MG2a-3 LO-MG2a-7 LO-MG2a-9				7×10^9 3×10^9 5.1×10^9 2.5×10^9				
LO-MG2b-1 LO-MG2b-2					3×10^8 1×10^{10}			
LO-MG3-7 LO-MG3-13						2×10^{10} 6.8×10^9		

As determined by the techniques of (1) Van Heymingen et al. J. Immunol. Methods 1983, 62: 147;(2) kindly given by Dr J. Van Snick;(3) Purified as described in Bazin and Malet,Immunology 1969, 17: 345;(4) Potter, Physiol.Rev. 1972, 52: 631;(5) ATCC 141;(6) Finkelman et al., J. Immunol. 1981, 126: 680.

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Detection and Quantification of MOUSE Ig

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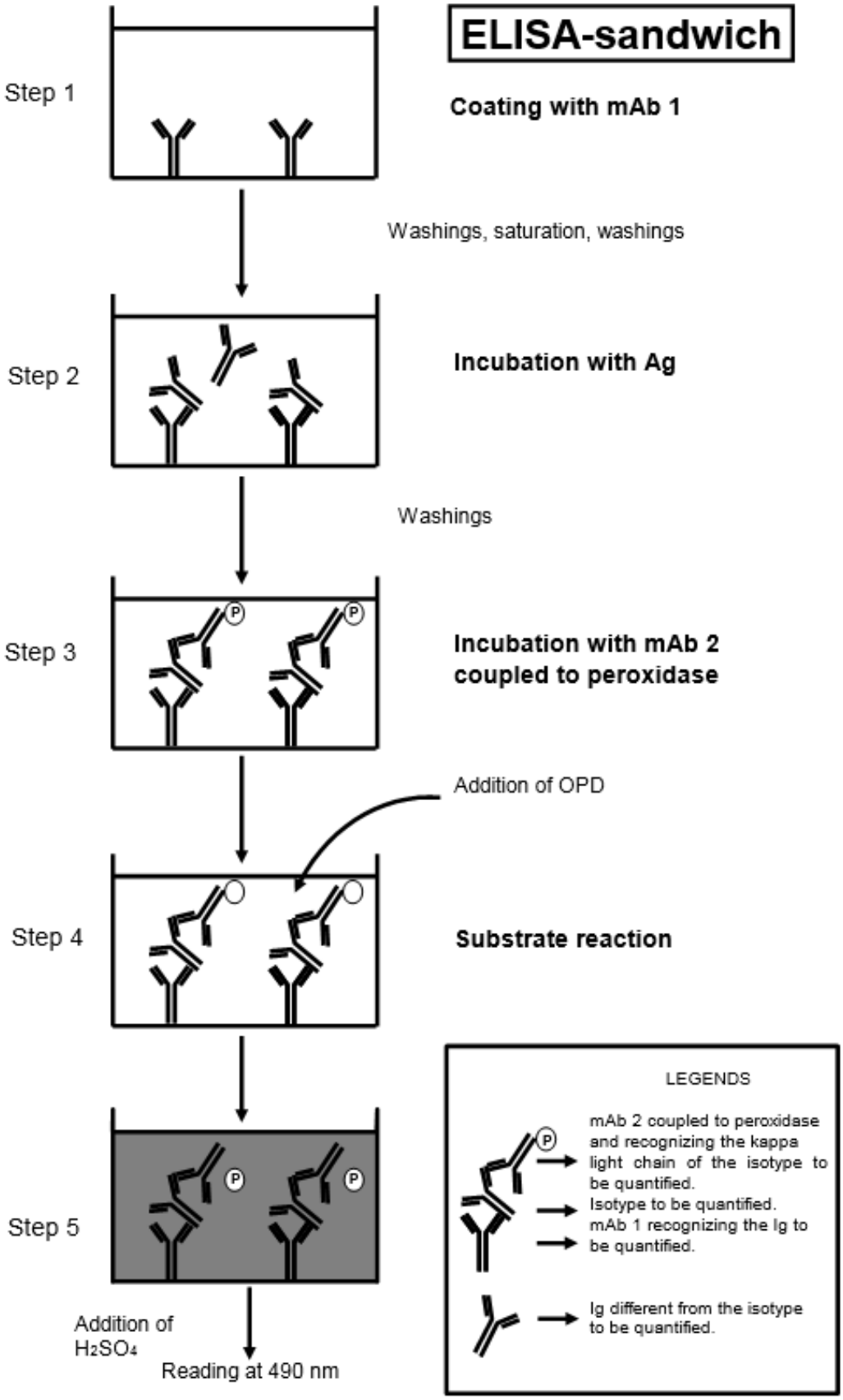

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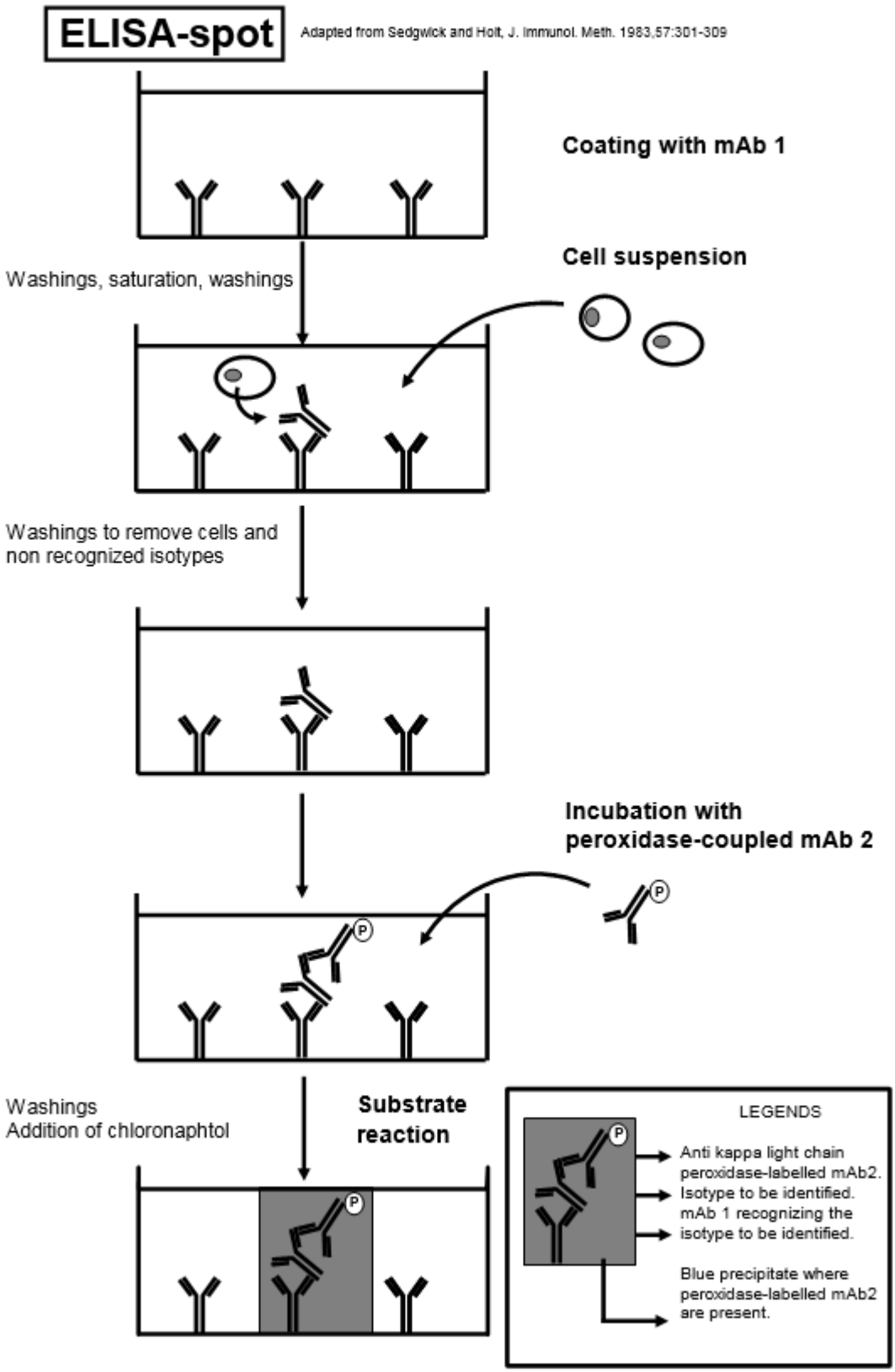

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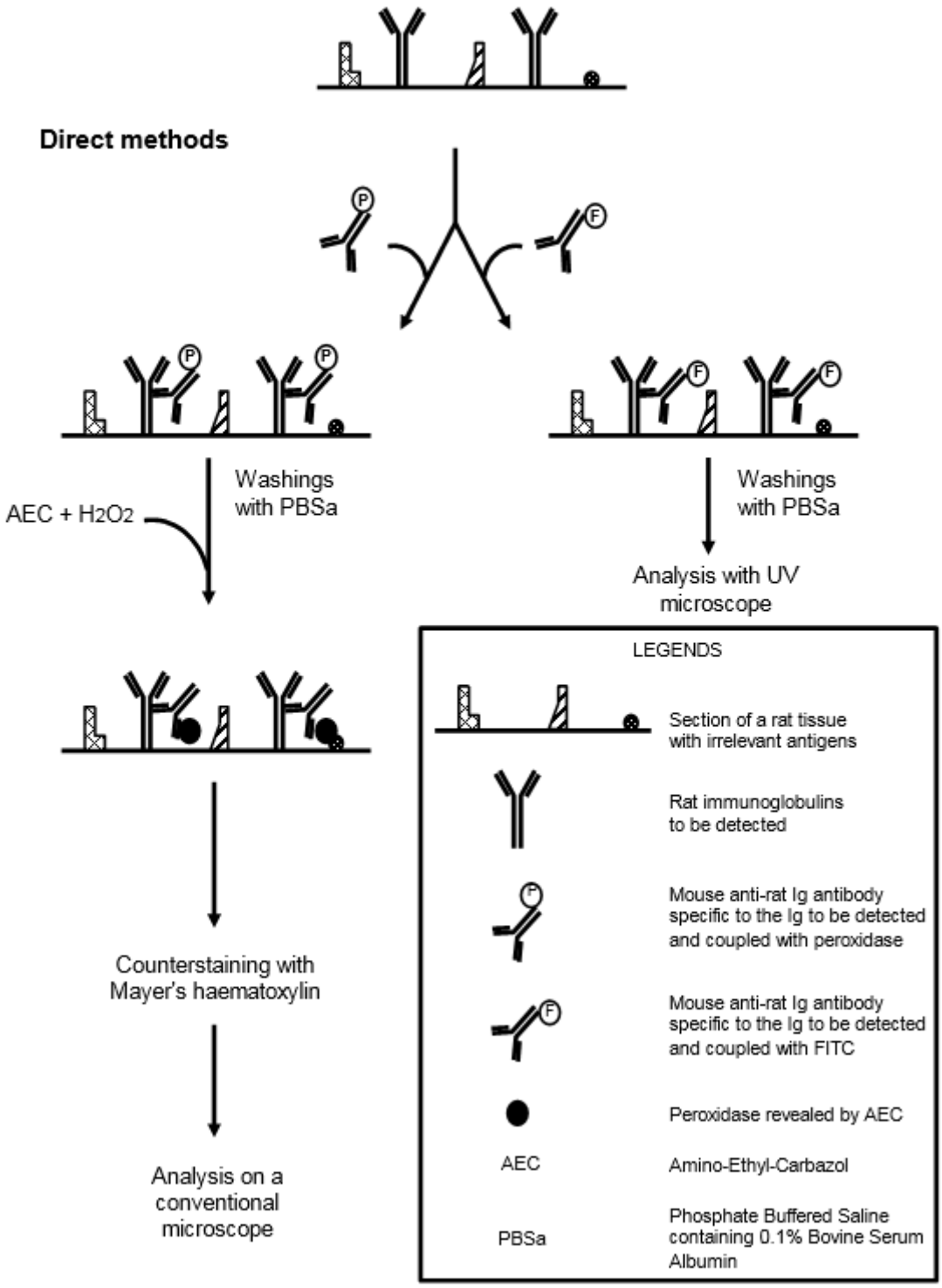
Immunohistolocalisation (1)



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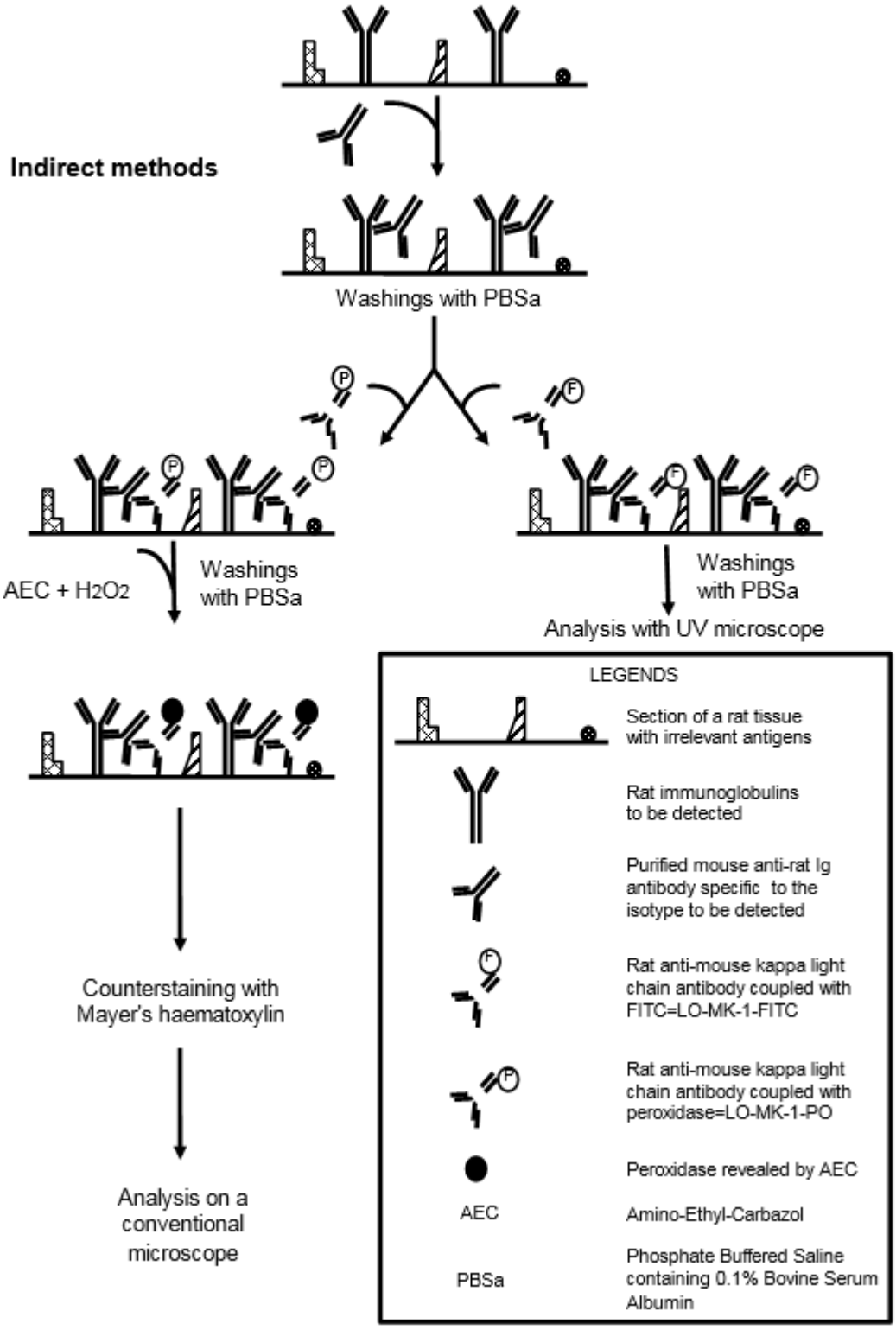
Immunohistolocalisation (2)



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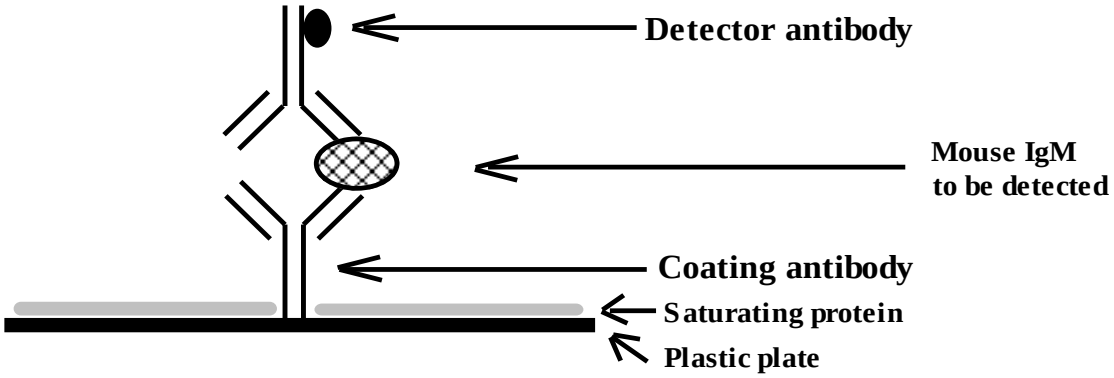
Detection and Quantification of **MOUSE IgM** by ELISA



	Sandwich ELISA assays			
	Option 1	**Option 2**	**Option 3**	**Option 4**
Detector antibodies	**LO-MM-9**	LO-MM-8	LO-MM-8	LO-MK-1*
Capture antibodies	**LO-MM-3**	LO-MM-3	LO-MM-9	LO-MM-3
Standard	MADNP-5			

Option 1 is recommended

Assay conditions

- **Coated antibody:** LO-MM-3 at 5 µg/ml (in a carbonate/bicarbonate buffer pH 9.5)
- **Saturating protein:** 5% lyophilized skimmed milk or some other proteins
- **Detector antibody:** LO-MM-9 conjugated to biotin and then streptavidin-peroxidase (or any other detection system) or LO-MM-8 conjugated to peroxidase
- **Normal range of detectable mouse IgM concentrations in this assay:** 2→0.1 µg/ml

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*Remark: Only kappa mouse IgM molecules are detected. In normal serum, mouse Ig molecules bearing kappa light chain represent about 95% of the total.

For each batch of monoclonal antibodies, all details of the ELISA controls are given in the quality control sheet.

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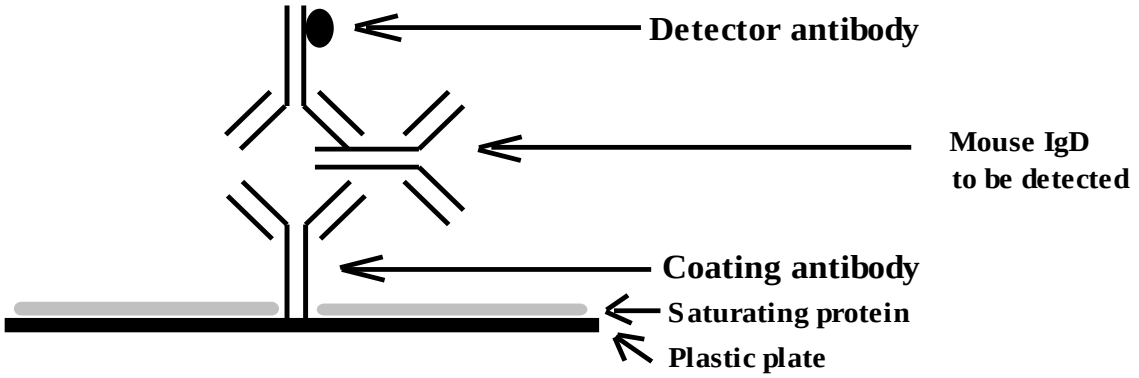
Detection and Quantification of **MOUSE IgD** by ELISA



	Sandwich ELISA assays	
	Option 1	**Option 2**
Detector antibodies	**LO-MD-8**	LO-MK-1*
Capture antibodies	**LO-MD-6**	LO-MD-6

Option 1 is recommended

Assay conditions

- **Coated antibody:** LO-MD-6 at 5 µg/ml (in carbonate/bicarbonate buffer pH 9.5)
- **Saturating protein:** lyophilized skimmed milk or some other proteins
- **Detector antibody:** LO-MD-8 conjugated to biotin and then streptavidin-peroxidase (or any other detection system)
- **Normal range of detectable mouse IgD concentrations in this assay:** 2→0.1 µg/ml

---

*Remark: Only kappa mouse IgD molecules are detected. In normal serum, mouse Ig molecules bearing kappa light chain represent about 95% of the total.

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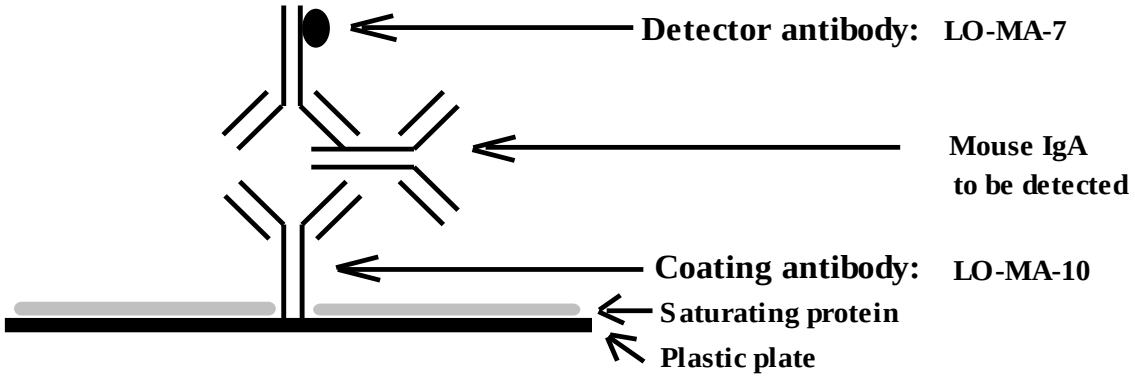
Detection and Quantification of **MOUSE IgA** by ELISA



	Sandwich ELISA assays	
	Option 1	**Option 2**
Detector antibodies	**LO-MA-7**	LO-MK-1*
Capture antibodies	**LO-MA-10**	LO-MA-10
Standards	Upon request	

Option 1 is recommended

Assay conditions

- **Coated antibody:** LO-MA-10 at 5 µg/ml (in carbonate/bicarbonate buffer pH 9.5)
- **Saturating protein:** lyophilized skimmed milk or some other proteins
- **Detector antibody:** LO-MA-7 conjugated to biotin and then streptavidin-peroxidase (or any other detection system)
- **Normal range of detectable mouse IgA concentrations in this assay:** 2→0.1 µg/ml

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*Remark: Only kappa mouse IgA molecules are detected. In normal serum, mouse Ig molecules bearing kappa light chain represent about 95% of the total.

For each batch of monoclonal antibodies, all details of the ELISA controls are given in the quality control sheet.

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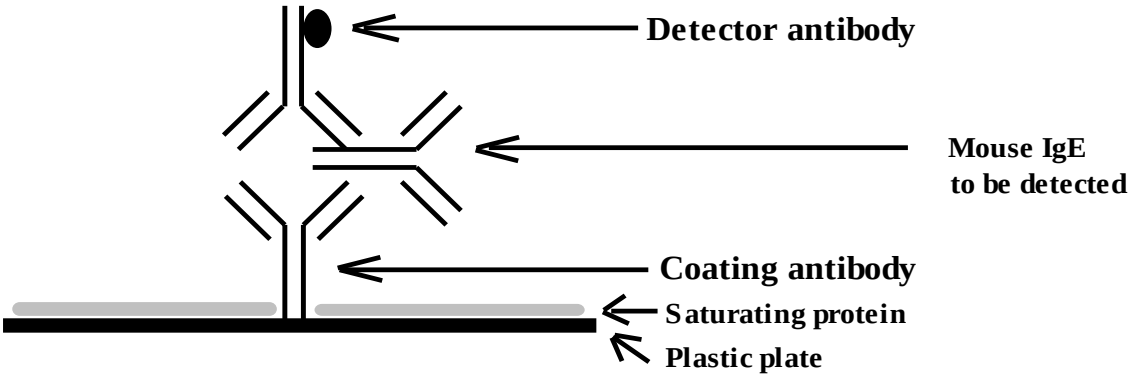
Detection and Quantification of **MOUSE IgE** by ELISA



	Sandwich ELISA assays	
	Option 1	**Option 2**
Detector antibodies	**LO-ME-2**	LO-MK-1*
Capture antibodies	**LO-ME-3**	LO-ME-3

Option 1 is recommended

Assay conditions

- **Coated antibody:** LO-ME-3 at 5 µg/ml (in carbonate/bicarbonate buffer pH 9.5)
- **Saturating protein:** lyophilized skimmed milk or some other proteins
- **Detector antibody:** LO-ME-2 conjugated to peroxidase or biotin and then streptavidin-peroxidase (or any other detection system)
- **Normal range of detectable mouse IgE concentrations in this assay:** 2→0.1 µg/ml

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*Remark: Only kappa mouse IgE molecules are detected. In normal serum, mouse Ig molecules bearing kappa light chain represent about 95% of the total.

For each batch of monoclonal antibodies, all details of the ELISA controls are given in the quality control sheet.

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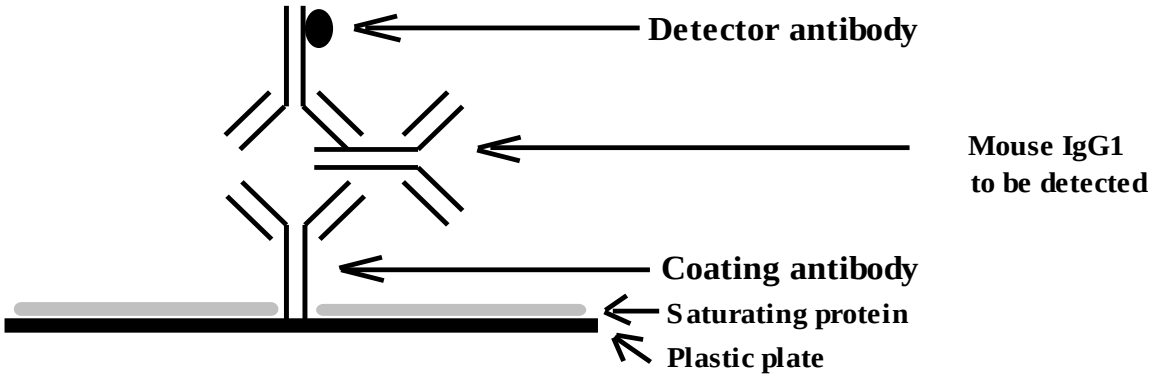
Detection and Quantification of **MOUSE IgG1** by ELISA



	Option 1	Option 2
Detector antibodies	**LO-MG1-2**	LO-MK-1*
Capture antibodies	**LO-MG1-13**	LO-MG1-13
Standard	MADNP-1	

Option 1 is recommended

Assay conditions

- **Coated antibody:** LO-MG1-13 at 5 µg/ml (in carbonate/bicarbonate buffer pH 9.5)
- **Saturating protein:** lyophilized skimmed milk or some other proteins
- **Detector antibody:** LO-MG1-2 conjugated to peroxidase or biotin and then streptavidin-peroxidase (or any other detection system)
- **Normal range of detectable mouse IgG1 concentrations in this assay:** 2→0.1 µg/ml

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*Remark: Only kappa mouse IgG1 molecules are detected. In normal serum, mouse Ig molecules bearing kappa light chain represent about 95% of the total.

For each batch of monoclonal antibodies, all details of the ELISA controls are given in the quality control sheet.

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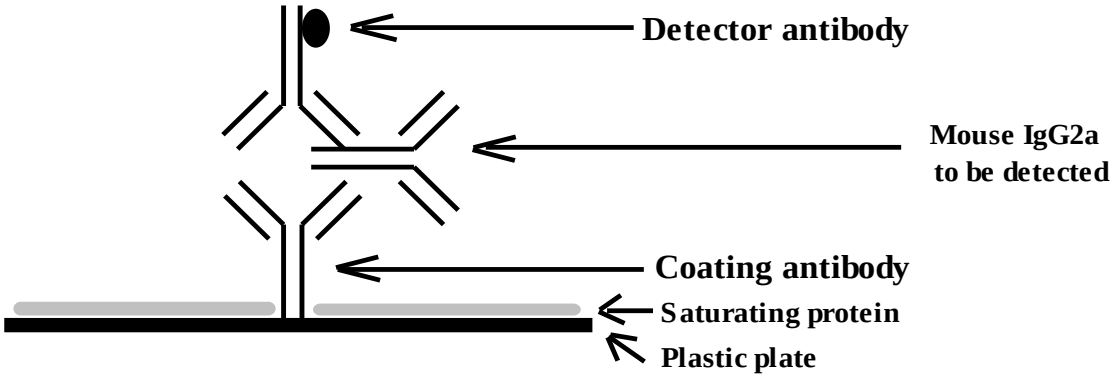
Detection and Quantification of **MOUSE IgG2a** by ELISA



	Sandwich ELISA assays			
	Option 1	**Option 2**	**Option 3**	**Option 4**
Detector antibodies	**LO-MG2a-3**	LO-MG2a-2	LO-MG2a-7	LO-MK-1*
Capture antibodies	**LO-MG2a-7**	LO-MG2a-7	LO-MG2a-9	LO-MG2a-9
Standard	MADNP-2			

Option 1 is recommended

Assay conditions

- **Coated antibody:** LO-MG2a-7and LO-MG2a-9 at 5 µg/ml (in carbonate/bicarbonate buffer pH 9.5)
- **Saturating protein:** lyophilized skimmed milk or some other proteins
- **Detector antibody:** LO-MG2a-2 or LO-MG2a-3 or LO-MG2a-7 conjugated to peroxidase or biotin and then streptavidin-peroxidase (or any other detection system)
- **Normal range of detectable mouse IgG2a concentrations in this assay:** 2→0.1 µg/ml

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*Remark: Only kappa mouse IgG2a molecules are detected. In normal serum, mouse Ig molecules bearing kappa light chain represent about 95% of the total.

For each batch of monoclonal antibodies, all details of the ELISA controls are given in the quality control sheet.

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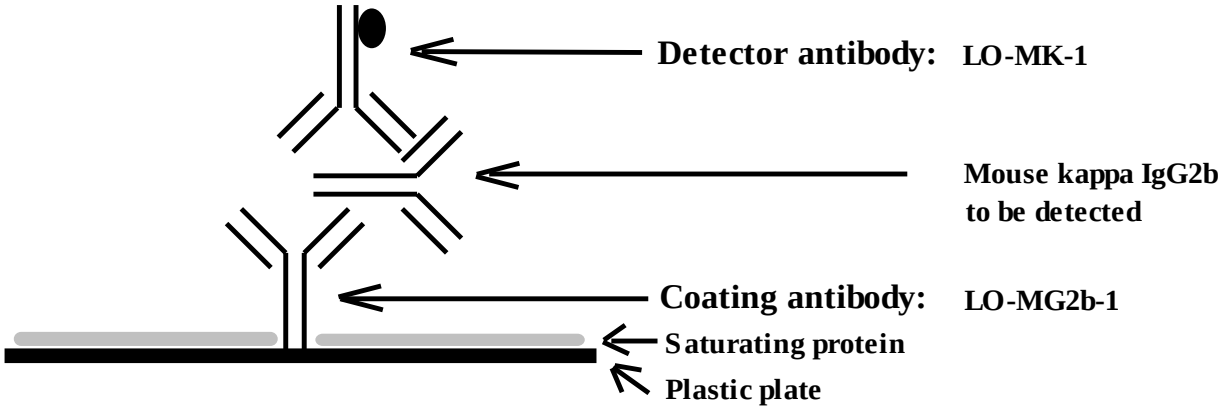
Detection and Quantification of **MOUSE IgG2b** by ELISA



- LO-MG2b-1 and LO-MK-1 can be used in Sandwich ELISA.
- LO-MG2b-1 coats on plastic plate.
- Standard: MADNP-3

Assay conditions

- **Coated antibody:** LO-MG2b-1 at 10 µg/ml (in carbonate/bicarbonate buffer pH 9.5)
- **Saturating protein:** lyophilized skimmed milk or some other proteins
- **Detector antibody:** LO-MK-1 conjugated to peroxidase or biotin and then streptavidin-peroxidase (or any other detection system)
- **Normal range of detectable mouse IgG2b concentrations in this assay:** 2→0.1µg/ml

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Remark: Only kappa mouse IgG2b molecules are detected. In normal serum, mouse Ig molecules bearing kappa light chain represent about 95% of the total.

For each batch of monoclonal antibodies, all details of the ELISA controls are given in the quality control sheet.

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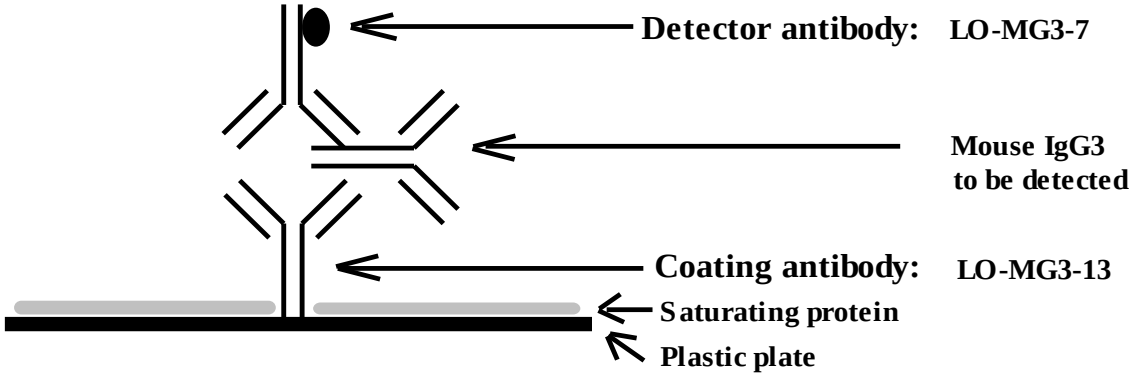
Detection and Quantification of **MOUSE IgG3** by ELISA



	Option 1	Option 2
Detector antibodies	**LO-MG3-7**	LO-MK-1*
Capture antibodies	**LO-MG3-13**	LO-MG3-13
Standard	MADNP-4	

Option 1 is recommended

Assay conditions

- **Coated antibody:** LO-MG3-13 at 5 µg/ml (in carbonate/bicarbonate buffer pH 9.5)
- **Saturating protein:** lyophilized skimmed milk or some other proteins
- **Detector antibody:** LO-MG3-7 conjugated to peroxidase or biotin and then streptavidin-peroxidase (or any other detection system)
- **Normal range of detectable mouse IgG3 concentrations in this assay:** 2→0.1 µg/ml

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Remark: Only kappa mouse IgG3 molecules are detected. In normal serum, mouse Ig molecules bearing kappa light chain represent about 95% of the total.

For each batch of monoclonal antibodies, all details of the ELISA controls are given in the quality control sheet

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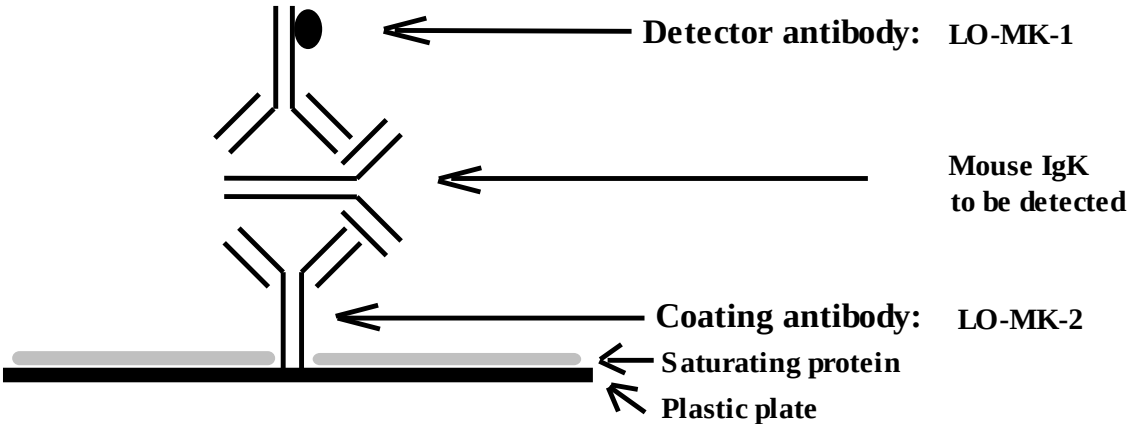
Detection and Quantification of **MOUSE IgK** by ELISA



- LO-MK-1 and LO-MK-2 do not bind to the same epitope(s) of mouse Ig.
- LO-MK-2 coats very well on plastic plate.
- Standards: upon request

Assay conditions

- **Coated antibody:** LO-MK-2 at 5 µg/ml (in carbonate/bicarbonate buffer pH 9.5)
- **Saturating protein:** lyophilized skimmed milk or some other proteins
- **Detector antibody:** LO-MK-1 conjugated to peroxidase or biotin and then streptavidin-peroxidase (or any other detection system)
- **Normal range of detectable mouse Ig concentrations in this assay:** 2→0.6 µg/ml

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Technique of purification of **MOUSE Ig** by Immunoaffinity chromatography

"x" ml of serum or ascitic fluid from BALB/c mice bearing or not a hybridoma are applied at a rate of about 2 ml/min at room temperature to a column of Sepharose-4B (Pharmacia, Belgium) on which "y" mg of rat monoAb anti-mouse Ig has been immobilized. The column is washed with 100-120 ml of phosphate buffered saline (PBS), then 100 ml of PBS containing 2.5 M NaCl, and then again at normal salinity with 100 ml of PBS. Mouse immunoglobulins are eluted by decreasing the pH with glycin HCL 0.1 M + 0.15 M NaCl buffer at pH 2.8*. The eluted fractions are neutralized as rapidly as possible after the elution with glycin NaOH buffer (0.1 M, pH 8.6) (Bazin et al., 1986; Cormont et al., 1986).

Examples of capacity of monoAb immunoaffinity column can be found in Bazin and Malache (1986).

For example, with the present experimental conditions, they were of 0.2 mg of mouse IgM for 1 mg of LO-MM-9 coupled to Sepharose-4B and 1 mg of mouse IgG1 for 1 mg of LO-MG1-2 coupled to Sepharose-4B.

*Depending on the rat monoclonal antibodies, the pH of the eluting buffer could be from pH 2.8 to pH 4.5.

REFERENCES:

BAZIN H., CORMONT F., DE CLERCQ L. Purification of rat monoclonal antibodies. Methods in Enzymology 1986, 121, 638-652.

CORMONT F., MANOUVRIEZ P., DE CLERCQ L., BAZIN H. The use of rat monoclonal antibodies to characterize, quantify and purify polyclonal or monoclonal mouse IgM. Methods in Enzymology 1986, 121, 622-631.

BAZIN H., MALACHE J.M. Rat (and mouse) monoclonal antibodies.V.A simple automated technique of antigen purification by immunoaffinity chromatography. J. Immunol. Methods 1986, 88, 19-24.

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IMMUNOAFFINITY PURIFICATION OF MOUSE IMMUNOGLOBULINS BY RAT MAb: INDICATIVE VALUES OF ELUTION (pH or ionic strength)

MAb Sepharose 4B	Elution buffer NaCl 2.5 M pH 7.2	Elution buffer NaCl 0.9 M pH 4.5	Elution buffer NaCl 0.9 M pH 3.8	Elution buffer NaCl 0.9 M pH 2.8
LO-MK-2	0	0	X	XX
LO-MM-9	0	0	XX	XX
LO-MG1-2	0	0	X	XX
LO-MG2a-3	0	0	XX	XX
LO-MG2b-2	0	0	XX	XX

0: no elution; X: partial elution; XX: complete elution.

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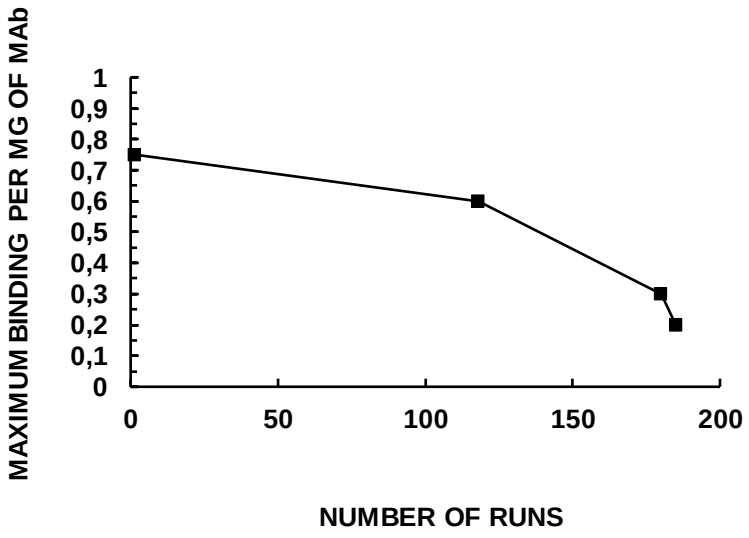
EXAMPLES OF BINDING CAPACITIES OF MOUSE IMMUNOGLOBULIN BY RAT MAb

MAb-Sepharose 4B	Number of runs already done with the column	Capacity (in mg) of binding per mg of MAb
LO-MK-2	2	0.45
LO-MM-9	38	0.2
LO-MG1-2	26	1.0
LO-MG2a-3	28	0.55
LO-MG2b-2	28	0.41

Immunoglobulin binding capacity in function of the number of runs (example taken in



Bazin and Malache, J. Immunol. Methods 1986, 88: 19-24 Monoclonal antibody: MARK-1). Most of the rat MAbs anti-mouse Ig isotypes seem to have a rather similar stability.

dianova GmbH ● Warburgstrasse 45 ● 20354 Hamburg, Germany
Phone: +49 (0)40 45 06 70 ● Email: info@dianova.de● www.dianova.com

Immunoglobulin to be purified	Column	1st peak (fall through)	2nd peak (eluted with acidic buffer)
5 ml of PB-1 BALB/c IgM secreting plasmacytoma mouse ascitic fluid	30 mg of LO-MM-9 (rat MAb anti-mouse IgM) coupled to 1.5 g of Sepharose 4B	Mouse ascitic fluid without IgM	7.8 mg of mouse PB-1 and polyclonal IgM
10 ml MARD-3 BALB/c IgG1 secreting MAb mouse ascitic fluid	25 mg of LO-MG1-2 (rat MAb anti-mouse IgG1) coupled to 1.0 g of Sepharose-4B	Mouse ascitic fluid without IgG1	20 mg of mouse MARD-3 and polyclonal IgG1
5 ml of MOPC-173 BALB/c IgG2a secreting plasmacytoma mouse ascitic fluid	25 mg of LO-MG2a-3 (rat MAb anti-mouse IgG2a) coupled to 1.0 g of Sepharose-4B	Mouse ascitic fluid without IgG2a	17.2 mg of mouse MOPC-173 and polyclonal IgG2a
7 ml of C1907 B3* BALB/c IgG2b secreting plasmacytoma mouse ascitic fluid	50 mg of LO-MG2b-2 (rat MAb anti-mouse IgG2b) coupled to 2.0 g of Sepharose-4B	Mouse ascitic fluid without IgG2b	21.9 mg of mouse C1907 B3 and polyclonal IgG2b
7.5 ml of MOPC-173 BALB/c IgG2a kappa secreting plasmacytoma mouse ascites	35 mg of LO-MK-2 (rat MAb anti-mouse Ig kappa) coupled to 2.0 g of Sepharose-4B	Mouse ascitic fluid without kappa L-chain immunoglobulin	12.4 mg of mouse MOPC-173 and polyclonal Ig kappa
45 ml of normal mouse ascitif fluid	30 mg of LO-MM-9 (rat MAb anti-mouse IgM) coupled to 1.5 g of Sepharose-4B	Normal mouse ascitic fluid without IgM	3.7 mg of mouse polyclonal IgM
30 ml of normal mouse ascitic fluid	25 mg of LO-MG1-2 (rat MAb anti-mouse IgG1) coupled to 1.0 g of Sepharose-4B	Normal mouse ascitic fluid without IgG1	16.0 mg of mouse polyclonal IgG1
25 ml of normal mouse ascitic fluid	25 mg of LO-MG2a-3 (rat MAb anti-mouse IgG2a) coupled to 1.0 g of Sepharose-4B	Normal mouse ascitic fluid without IgG2a	9.1 mg of mouse polyclonal IgG2a
25 ml of normal mouse ascitic fluid	50 mg of LO-MG2b-2 (rat MAb anti-mouse IgG2b) coupled to 2.0 g of Sepharose-4B	Normal mouse ascitic fluid without IgG2b	7.5 mg of mouse polyclonal IgG2b
15 ml of normal mouse ascitic fluid	35 mg of LO-MK-2 (rat MAb anti-mouse Ig kappa) coupled to 2.0 g of Sepharose-4B	Normal mouse ascitic fluid without kappa L-chain immunoglobulin	12.0 mg of mouse polyclonal Ig kappa.

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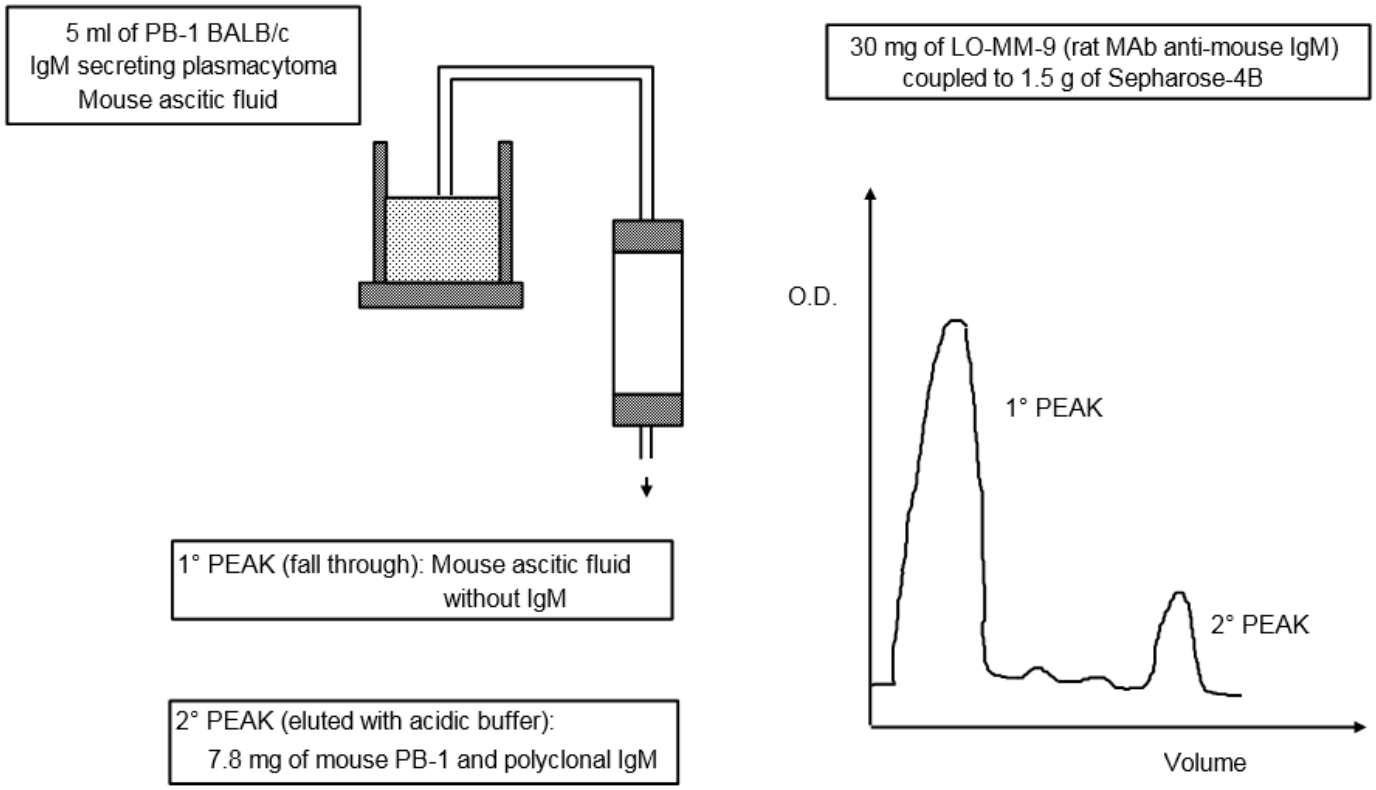
*Kindly provided by Dr J. Van Snick (UCL)

EXAMPLE

Purification by Immunoaffinity of IgM MAb



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Cross-Reactivity Test of Rat Anti-Mouse Ig Mab with Human Immunoglobulin

Experimental conditions

EIA plates (NUNC, Denmark, Cat. No. 4-69914) were coated overnight at 4°C with 10 µg/ml polyclonal human IgG (Gamma 16, Institut Mérieux, France) in 0.01M borate buffer at pH 9.6. Remaining binding sites were saturated with 0.5% gelatin in PBS 0.1% Tween. MAbs were revealed with peroxidase labeled Rabbit anti-Rat Ig antibodies. All incubations were done for 1 hour at room temperature and washings made with PBS 0.1% Tween. A signal was considered as positive if twice the background O.D. obtained by incubation with P.O. labeled antibody without previous incubation with rat antibodies.

Results

Antibody	Concentration at which a positive signal appears	Cross-reaction with human Ig
LO-MG1-2	< 15 ng/ml	Yes
LO-MG1-13	> 1200 ng/ml	Yes*
LO-MG2a-2	> 62500 ng/ml	No
LO-MG2a-3	not at 250000 ng/ml	No
LO-MG2b-2	not at 250000 ng/ml	No
LO-MG3-7	> 15600 ng/ml	No

*Yes, but sometimes, can be avoided by careful manipulations.

When labeled with P.O., these MAbs can be used at concentrations of 125-500 ng/ml

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