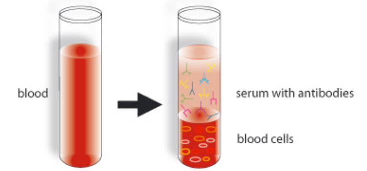
Protocol 509_IgE ELISA mouse serum

Sample collection:
Collect blood into serum tube (brown cap)
Centrifuge serum for 10' at 10.000 g to separate cells, fibrinogen and other proteins involved in coagulation from serum (supernatant)
Store sample at -20˚C until performing ELISA



Method:
Day 0

COATING (up to 7 samples per 96 well plate)

1. Dilute **anti-IgE Ab (6HD5)** in ELISA coating buffer to 5 μg/ml
   Calculate 5 ml coating buffer/plate
2. Add 50 μl/well to ELISA plates (96 well, flat bottom,)
3. Wrap in saran, O/N at 4°C

Day 1

1. Flick off unbound coating antibody
2. Wash plate **3×** with ELISA washing buffer

BLOCKING

3. Block plates by adding 100 μl/well 5% BSA, PBS
4. Incubate **2 h at RT**

LOADING SAMPLES

5. Flick off blocking solution, no need to wash here
6. Titrate samples on plates (see plate layout below)
   a. Put 110μl 2.5 % BSA in PBS into A2-A12
   b. Put 55 μl 2.5 % BSA in PBS into B1-H12
   c. Put 165 μl **standard IgE at 5000 ng/ml** diluted in 2.5 % BSA in PBS into A1, titrate 55μl to A12 (1:3 titration)
      for standard in row A titrate to column 12
   d. Put 55 μl serum samples 1-7 into B1-H1
   e. Titrate for all rows (B-H) 55 μl across the plate to column 11, discard last 55 μl (serum 1:2 titration).
7. Incubate **2h at RT**
8. Wash plates **3×** with ELISA washing buffer

SECONDARY ANTIBODY

9. Add **50 μl/well of secondary ab: anti-IgE(RIE4)-biotin** diluted in PBS 2.5% BSA to 2.5 μg/ml
10. Incubate **1.5h at RT** (or O/N at 4°C)
11. Wash plates 3× with ELISA washing buffer

STREPTAVIDIN

12. Add **100 μl/well streptavidin-HRPO** -Pat no 890803 R&D systems- (horseradish peroxidase) at 1 μg/ml (1:500 in PBS 2.5% BSA)
13. Incubate **max 1 h at RT** (35 min to max 1 h)
14. Wash plates 3× with ELISA washing buffer

DEVELOPING PLATE

15. Add **100 μl/well Reaction buffer**

16. Incubate at RT until color reaction (don't leave too long **(~10-20 min**) as background can get strong)
17. Read reaction after ~15 min in ELISA reader at 415 nm. Standard~1.0, blank~0.1

Principle:

Buffers:

Substrate buffer:
0.1 M NaH_2PO_4, pH 4 (13.8 g NaH_2PO_4 made up to 1000 ml)

Reaction Buffer:
Add 1 mg ABTS (Boehringer) and 5 μl H_2O_2 just before use to 10 ml Substrate buffer

Washing buffer:
10 L of PBS
0.05% Tween 20 (Polyoxyethylene 20) – 5 ml –

2.5% BSA in PBS:
25 g BSA (Bovine Serum Albumin) in 1 L PBS. Stirr it and then sterile filter. Keep in fridge

5% BSA in PBS:
50 g BSA in 1L PBS. Stirr it and then sterile filter. Keep in fridge.

Plate layout:

5000	**1666.67**	**555.56**	**185.19**	**61.73**	**20.58**	**6.86**	**2.29**	**0.76**	**0.25**	**0.08**	**0.03**
S1											Blank
S2											
S3											
S4											

S5											
S6											
S7											

PLATE READING and analysis with MPM III 1.8

- ELISA reader is on G floor (Switch on, password 00000)
- Open MPM III software on computer next to ELISA reader
- File>New experiment>Read plate
- Reading mode: Endpoint; Mix time(s): 3: mixing speed: L
- click “start read”
- after plate has been read check whether standard curve is S-shaped:
- file>open protocol>IgE protocol
- then go to “curve fit” and check curve
  - if it it still linear, wait a few minutes longer and read again
  - if the curve fit is good: r2 0.9-1, save file!

To analyse:

- Open data file and IgE protocol
- go to: “Report”
- use value Y=…/0.345/… to get the value of your sample in the titration series that fits the linear standard curve and therefore reflects the true IgE level best
- the IgE concentration is given in **ng/mL**

File:

IgE protocol for the above plate layout is named: IgE protocol and saved as 510_IgE protocol

Number of plates	Reagent	stock conc		end conc		Vf (ml)	Vi (µl) ?
1	Coating antibody 6HD5	5,43	mg/ml	5	µg/ml	5	4,60405157
2		5,43	mg/ml	5	µg/ml	10	9,20810313
3		5,43	mg/ml	5	µg/ml	15	13,8121547
4		5,43	mg/ml	5	µg/ml	20	18,4162063
5		5,43	mg/ml	5	µg/ml	25	23,0202578
6		5,43	mg/ml	5	µg/ml	30	27,6243094
1	Secondary antibody RIE-4	1,3	mg/ml	2,5	µg/ml	5	9,61538462
2		1,3	mg/ml	2,5	µg/ml	10	19,2307692
3		1,3	mg/ml	2,5	µg/ml	15	28,8461538
4		1,3	mg/ml	2,5	µg/ml	20	38,4615385
5		1,3	mg/ml	2,5	µg/ml	25	48,0769231
6		1,3	mg/ml	2,5	µg/ml	30	57,6923077