Protocol 505_ELISA protocol for detection of mouse interleukin-2 (IL-2)

Reagents:

- Coating buffer: 0.1M $NaHCO_3$, pH 8.2 (84.01 g/mol → 0.1 M. = 4.2 g in 500 ml)
- Washing buffer: PBS-Tween, 0.05% Tween-20 in PBS (0.5ml Tween-20 in 1000ml PBS)
  For 10L tank: 10L ddH2O, 10x PBS powder, 5ml Tween-20, SHAKE WELL!
- Blocking solution: 2% BSA in PBS. Stir for ca 30min and STERILE FILTER!
- Substrate buffer: 0.1 M sodium phosphate monobasic NaH_2PO_4, pH 4 (13.8g NaH_2PO_4 made up to 1000ml or 14.6g sodium phosphate monobasic monohydrate $NaH_2PO_4.H_2O$).
- Diluent for samples and antibodies etc: 0.5% BSA in PBS. Stir for ca 30min and STERILE FILTER!
- ELISA plates: MAXISORP immunoplates (Nunc 456537)
- HRP substrate: 10ml (per plate) Substrate buffer, 1mg ABTS, 5µl H_2O_2 (prepare just before use)

Antibodies:

Capture antibody: JES6-1A12 (BD 554424, 0.5mg/ml) at 4µg/ml
Detection antibody: JES6-5H4-Biotin (BD 554426, 0.5mg/ml) at 1µg/ml

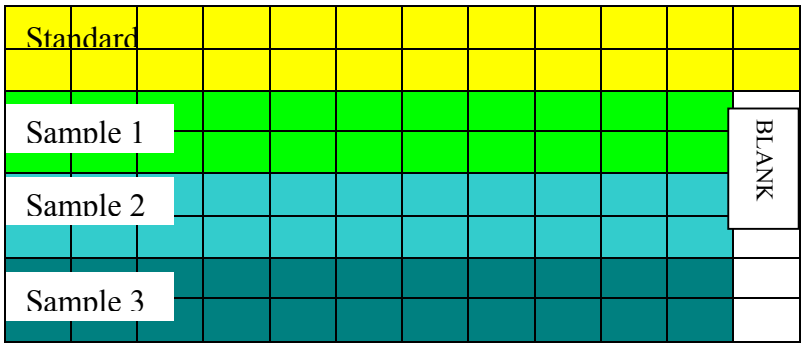
Standards:

Recombinant mouse IL-2 (BD 550069):
Comes as 20µg in 100µl (200µg/ml). BD stock vial was diluted and stored at -80°C as 10µl aliquots at 50µg/ml.
Use recombinant mouse IL-2 stock solutions at 50µg/ml (stored at -80°C) to prepare 50ng/ml personal stocks by diluting in 0.5% BSA (keep at -20°C, the one tube being in use can be kept at 4°C).
Construct a standard curve (in duplicate) in a clean separate soft 96-well plate by 2-fold serially diluting 100µl into 100µl diluent starting **at a top concentration of 10ng/ml (1:5 of the 50ng/ml stock solution).**

Method:

1. Dilute capture antibody to 4µg/ml in coating buffer (you need ca 5ml per plate).
2. Pipette 50µl of the capture antibody solution into each well of a 96-well flat-bottom ELISA plate using a multi-channel pipette. Remember to include enough plates for all the experimental supernatants and for the standards.
3. Incubate 6h to overnight at 4°C. Wrap plate with cling film or parafilm.
4. Wash 3x with PBS-Tween.
5. Block non-specific binding sites by adding 200µl/well of blocking solution (2% BSA) for 2h at room temp.
6. Wash 3x with PBS-Tween.

7. Prepare two-fold serial dilutions of your samples in diluent in a separate soft 96well plate. Add 50 µl of the diluted samples AND standards to the respective wells (include duplicates for the standards on EVERY plate).



8. Incubate 2h (longer to increase sensitivity) at room temperature or, for higher sensitivity, overnight at 4°C. Wrap plate with cling film.

9. Wash 6x with PBS-Tween.

10. Add 100µl detection antibody at 1µg/ml. Incubate 1h at room temperature.

11. Wash 6x with PBS-Tween.

12. Add 100µl HRP-Streptavidin (1:1000 in 0.5% BSA). Incubate 45min at room temperature.

13. Wash 6x with PBS-Tween (followed by 2x distilled water).

14. Add 100µl substrate, cover plates, and incubate at room temperature for 10-15min. Read for the first time as soon as a nice green color develops in the standard wells.

15. Note:
The colour reaction can be stopped by addition of 100µl stop solution (0.05M NaF, 0.5M EDTA, 0.05M NaOH). This is NOT necessary if plates are measured immediately once the colour is nicely green.

16. Read A405. Blanks should NEVER be higher than 0.1. Do NOT trust values exceeding 1.2!

17. Plot the A405 of the standards against the cytokine concentration to obtain a standard curve. Try to use the linear part of the curve for accurate determinations (see separate protocol for software use).

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.150	0.922	0.597	0.306	0.164	0.099	0.081	0.069	0.052	0.070	0.067	0.050
B	1.106	0.894	0.587	0.319	0.162	0.106	0.085	0.085	0.099	0.054	0.057	0.057
C	1.121	1.028	0.741	0.434	0.217	0.155	0.087	0.078	0.098	0.076	0.057	0.057
D	1.151	1.016	0.756	0.464	0.247	0.173	0.089	0.104	0.089	0.081	0.072	0.060
E	1.094	0.943	0.643	0.377	0.191	0.142	0.077	0.074	0.077	0.077	0.074	0.066
F	0.235	0.141	0.092	0.130	0.078	0.096	0.076	0.067	0.066	0.068	0.066	0.066
G	0.203	0.111	0.120	0.225	0.098	0.064	0.067	0.059	0.070	0.061	0.063	0.090
H	0.231	0.109	0.138	0.092	0.076	0.063	0.062	0.066	0.064	0.063	0.064	0.069