

# Protocol



## ELISA for Mouse IgG

Version 1.0

**Background** This ELISA is useful for measuring mouse IgG concentrations in cell culture media or ascites fluid.

### **Procedure**

1. Coat 0.5  $\mu\text{g}$  of F(ab')<sub>2</sub> donkey anti-mouse IgG (715-006-151) in 200  $\mu\text{l}$  of 50 mM Na<sub>2</sub>CO<sub>3</sub>, pH 9.6 overnight at room temperature in the dark.
2. Remove coating solution and replace with 350  $\mu\text{l}$  of full PBS containing 0.5% IgG-free BSA (A-7638) and 0.05% Tween-20 (block buffer). Incubate for 1 hour with shaking at room temperature.
3. Wash three times quickly with PBS containing 0.05% tween-20.
4. Incubate with 200  $\mu\text{l}$  standards or samples, diluted in block buffer for 1 hour with shaking at room temperature. Use standards of Sigma mouse IgG, with top standard 10 ng/ml and doubling dilutions thereof down to 0.078 ng/ml.
5. Wash three times quickly with PBS containing 0.05% tween-20.
6. Incubate with 200  $\mu\text{l}$  of goat anti-mouse IgG peroxidase (A0168) diluted 1/10,000 in block buffer for 1 hour with shaking at room temperature.
7. Wash three times quickly with PBS containing 0.05% tween-20.
8. Incubate with 200  $\mu\text{l}$  K-blue substrate for 7 minutes. Stop with 50  $\mu\text{l}$  2M H<sub>2</sub>SO<sub>4</sub>.

### **KEY POINTS:**

- \* For the most accurate and reproducible ELISA always randomise your samples onto the plate, and do not wash starting from the same column every time.
- \* In most circumstances a very large dilution of your test sample will be required as this assay is very sensitive. I have shown that dilution of antibodies in cell culture supernatant gives a linear response, and that spiking purified mouse IgG into cell culture supernatant gives approximately 100% recovery as well.



### *Materials*

F(ab') <sub>2</sub> donkey anti-mouse IgG	Jackson ImmunoResearch	715-006-151
IgG-free BSA	Sigma	A-7638
Goat anti-mouse IgG peroxidase	Sigma	A-0168
K-blue substrate	Skybio	KB-176

Coating buffer: 50 mM Na<sub>2</sub>CO<sub>3</sub>, pH 9.6

PBS: 8 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.5 mM KH<sub>2</sub>PO<sub>4</sub>, 137 mM NaCl, 2.7 mM KCl, 0.9 mM CaCl<sub>2</sub>, 0.5 mM MgCl<sub>2</sub>, pH 7.2

### *Source References*

None

### *References where we have used this protocol*

None

### **DISCLAIMER**

**Please note that this protocol is provided for information only. While we believe it to be accurate, we do not warrant as to its validity or suitability for any particular purpose. In the event that you wish to use this protocol, we accept no liability for any direct or indirect losses howsoever caused. It is your responsibility to ensure that any use of this protocol is performed safely and in accordance with any local laws, regulations or guidelines applicable to you.**