

# Worksheet E2

## General Procedure for Indirect Sandwich ELISA using Streptavidin-Biotin Detection

### Note:

This method provides a general procedure for use with the majority of Biozol reagents. In some cases specific recommendations are provided on product datasheets, and this method should always be used in conjunction with product and batch specific information provided with each vial. Please note that a certain level of technical skill and immunological knowledge is required for the successful design and implementation of these techniques - these are guidelines only and may need to be adjusted for particular applications.

1. Coat microtitre plate wells with 100 µl of appropriate coating antibody, at a concentration of between 1-10 µg/ml in coating buffer.
2. Cover plate and incubate overnight at 4°C. Wash plate x3 in wash buffer.
3. Add 150 µl of blocking solution to each well. Incubate 60 minutes at 37°C. Wash x3 in wash buffer.

**N.B.** For most Biozol antibodies this step can be omitted without having any deleterious effects on the results obtained.

4. Add 100 µl of appropriately diluted samples to relevant wells. Ensure that appropriately diluted standards are included. Samples or standards should preferably be run in triplicate. Incubate overnight at 4°C. (Dilute samples and standards in wash buffer). Wash x3 in wash buffer.
5. Add 100 µl of appropriately diluted biotin conjugated detection antibody to each well (Dilute antibody in ELISA wash buffer). Incubate for 60 minutes at 37°C. Wash x3 in wash buffer.
6. Add 100 µl of appropriately diluted enzyme conjugated streptavidin to each well. Incubate for 60 minutes at 37°C. (Dilute in wash buffer). Wash x3 in wash buffer.
7. Add 100 µl of appropriate substrate solution to each well. Incubate at room temperature (in the dark if required) for 30 minutes, or until desired absorbance values are attained.
8. Read absorbance values at appropriate wavelength.

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**Recommended buffers:**

**Coating buffer:**

1.5 g Na<sub>2</sub>CO<sub>3</sub>, 2.93 g NaHCO<sub>3</sub>, 1 litre distilled water pH 9.6.

*For alternative coating buffer use BZL05188.*

**Blocking buffer:**

PBS containing 1% w/v bovine serum albumin (BSA).

*For alternative blocking buffer use ELISA BSA BLOCK (BZL05191), or ELISA ULTRABLOCK (BZL04925) or ELISA SYNBLOCK (BZL05195).*

**Wash buffer:**

PBS containing 0.05% v/v Tween 20.

*For alternative wash buffer use BZL04922.*

**FOR RESEARCH USE ONLY. NOT FOR THERAPEUTIC OR DIAGNOSTIC USE**

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