

## **Method guide for conjugating Smartbond Streptavidin to Horseradish Peroxidase RZ3 grade**

### **1.0 Precautionary Notes**

*Some of the materials are hazardous and should be handled with consideration of country specific safety regulations e.g. COSHH, OSHA.*

### **2.0 Reagents (to be prepared in purified water)**

- A. 1 mM Sodium Acetate pH 4.4
- B. 0.1 M Sodium Periodate (protect from light e.g. prepare in amber vial)
- C. 10 mM Sodium Carbonate pH 9.5
- D. 0.2 M Sodium Carbonate pH 9.5
- E. 4 mg/ml Sodium Borohydride (prepare just before use; refer 3.8).
- F. Phosphate Buffered Saline pH 7.2

### **3.0 Procedure**

- 3.1 Prepare a 4 mg/ml solution of Horseradish peroxidase (HRP) in Solution A.
- 3.2 Determine the amount of Solution B to add to the HRP solution by the following calculation:  

$$\text{Volume of Solution B to add (in mls)} = \text{Volume of HRP Solution (in mls)} \times 0.19$$
- 3.3 Add Solution B to the HRP solution and stir at room temperature for 20 minutes (protect from light).
- 3.4 Prepare a 5 mg/ml solution of Smartbond Streptavidin in Solution C (mix gently by inversion) i.e. at least 1/5<sup>th</sup> of the selected volume of the HRP solution (see example table below).
- 3.5 Add 20 µl of Solution D per ml of HRP stock solution prepared in step 3.1.
- 3.6 Determine the amount of Smartbond Streptavidin to add according to the following calculation:  

$$\text{Volume of Streptavidin to add (in mls)} = \text{Total Volume of Reaction Mixture (in mls)} \times 0.17$$
- 3.7 Add the calculated amount of Smartbond Streptavidin and stir for 2 hours at room temperature.
- 3.8 Prepare Solution E and add to the reaction mixture according to the following calculation:  

$$\text{Volume of Sodium Borohydride to add (in mls)} = \text{Total Volume of Reaction Mixture (in mls)} \times 0.05$$
- 3.9 Stir the mixture for 2 hours at 4 °C.
- 3.10 Separate conjugated from unconjugated material by size exclusion chromatography: first concentrate (if applicable) to a volume which represents 2 % of the column volume using an ultrafiltration spin concentrator e.g. for example, based on volumes in the table below, concentrate to ~9 ml for a 2.6 cm (diameter) x 85 cm (length) packed column of S-200 HR resin equilibrated in Solution F.
- 3.11 Perform chromatography (with Solution F) and collect the 'first' major eluting protein peak.
- 3.12 For long term storage mix the 'conjugate' pool with glycerol (to 50 % v/v) and store at -20 °C.

#### **Example:**

<b>Solution</b>	<b>Volume (ml)</b>
HRP (4 mg/ml)	25
Solution B	4.8
Solution D	0.5
Streptavidin (5 mg/ml)	5.2
Sodium Borohydride (4 mg/ml)	1.8

### **Technical Support**

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