

General Protocol for Immobilized Soybean Peroxidase (SBP-I) (Cat # 515-SS)

Background: Soybean Peroxidase (SBP) is immobilized into polyurethane foam. The immobilized SBP (SBP-I) foam can be form fitted. At Bio-Research Products (BRP), the continuous flow and batch process methods have been employed for several oxidations. For the continuous flow method, the foam is fitted within a column, while substrate, solvent, and peroxide pass through the column and is collected. The reaction and peroxide in the collection vessel can be monitored and adjusted during the reaction solution is flowing through the column and recycled until the reaction is complete. The batch method uses pieces of SBP-I in a vessel with the solvent and substrate; the system is often agitated by either magnetic or mechanical stirring. The peroxide can be introduced by a dropping funnel or peristaltic pump. During the course of a reaction for either method, the peroxide must be monitored; excess peroxide will inactivate the enzyme.

Continuous Flow Process

The columns typically used for protein chromatography can accommodate the SBP-I foam either by form fit or packing with cut foam. Prior to any reaction, the SBP-I foam should be equilibrated in the reaction solvent with at least 2 column volumes (CV). In Figure 1, the reaction mixture circulates through the column by a peristaltic pump. The collection vessel has peroxide (H_2O_2), solvent and substrate; the reaction can be monitored for H_2O_2 and reaction progress. If the reaction is incomplete, the pump can recycle the reaction mixture with fresh peroxide or substrate.

A typical 300 mL foam column will contain approximately 160-200 KU SBP. The ratio of peroxide to substrate is 1.5:1. Substrate concentrations can range between 10 to 50 mM for 500 mL of solvent. The solvent is typically a buffered solution with 0.2 M $CaCl_2$ at pH 2 to 11 depending on the substrate. For hydrophobic substrates, BRP has proprietary technology to enhance solubility of hydrophobic compounds, eliminating use of **any** organic solvent. Reaction progress can be monitored

by HPLC and peroxide concentration determined colorimetrically using the BRP Titanium Sulfate Reagent Kit. Reaction optimization should always be performed with the free SBP enzyme prior to using the immobilized SBP.

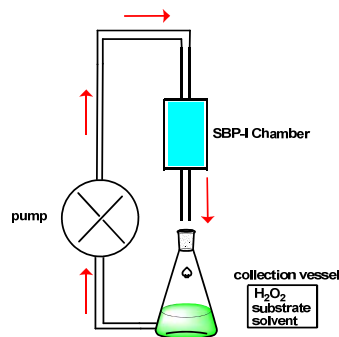


Figure 1: Continuous flow method consists of 3 major components: the SBP-I Column, Collection vessel, and pump, which are all connected through flow tubing indicated by the red arrows.

Batch Process

The batch process uses pieces of cut SBP-I foam in a reaction vessel containing the solvent and substrate. The H_2O_2 is delivered either by peristaltic pump or dropping funnel. Figures 2-5 illustrate batch reaction set-ups for general method applications. The SBP-I activity used depends on the scale of reaction by volume and molar concentration of substrate. A general reaction uses 5 g cut foam, containing

23.2 KU activity in 100 mL solvent (buffer), and 15-20 mM substrate. The ratio of peroxide to substrate is 1.5:1 and will be $1.5 \times 20 \text{ mM} = 30 \text{ mM}$. Additions should be slow and metered. The reaction must be monitored for peroxide concentration. Reaction progress can be monitored by HPLC, GC or any other method. For incomplete reaction, further addition of peroxide may be necessary. Reaction optimization

should always be performed with the free SBP enzyme prior to using the immobilized SBP.

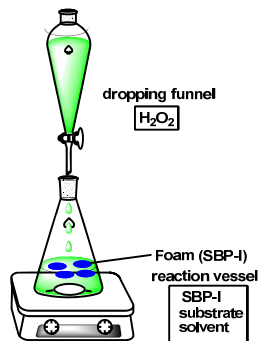


Figure 2: Batch process using H_2O_2 delivery from a dropping funnel to reaction vessel (Erlenmeyer flask). The reaction mixture is agitated by magnetic stirring.



Figure 3: Cut foam for batch process.



Figure 4: Cut foam in Erlenmeyer flasks for batch process.

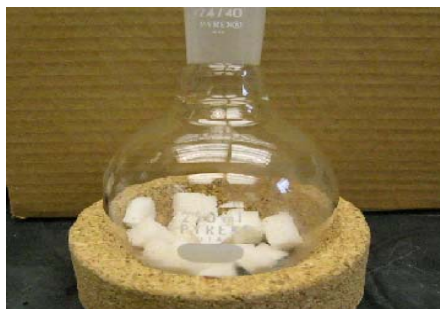


Figure 5: Cut foam in round bottom flask for batch process.