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## SIGMA INSOLUBLE ENZYMES

### Product Description

Sigma insoluble enzymes are produced by reacting the conventional "soluble" enzyme with an inert base that results in an insoluble compound retaining the activity of the original enzyme. Applications are almost unlimited. By selecting the "inert" base carefully, a highly active "resin" or "gel" can be produced with which the enzyme reaction can be quickly catalyzed by momentary contact with the substrate in a suitable medium. This can be carried out either in a batch slurry or even through a small column. In either case, the insoluble enzyme does not remain as a contaminant of the reaction mix. It can be readily filtered or centrifuged out of the batch slurry, or remain behind in the column. Many unstable enzymes seem to be much more stable in the insoluble form. In fact, Sigma insoluble enzymes can be reused many, many times.

Based upon preliminary studies, agarose seems to be the best base to use. It is quite inert in most systems and allows high activities per unit weight. Sigma already offers a long, growing list of the common "routine" enzymes on this agarose matrix.

We also offer a list of insoluble enzymes on polyacrylamide, acrylic beads and carboxymethyl cellulose. These matrices also seem to have useful applications, but because of their absorptive properties, require more discreet handling.

### Instructions for Use

#### I. Preparation

1a. If product form is lyophilized powder, suspend the required amount to 5-10 mg solid/ml water and allow brief hydration. (NOTE: For polyacrylamide insoluble enzymes, it is necessary to allow the suspended enzyme to stand at 2-8 °C for two hours. Then proceed with the filter/wash step.)

## Product Information

- 1b. If product form is suspension, gently mix then remove required amount for filter/wash step.
2. Filter and wash several times with water and/or your buffer to remove the suspension medium. (NOTE: The packaging medium may contain stabilizers, which could inhibit enzyme activity.)
3. Resuspend in the appropriate buffer. The enzyme is now ready for use.

#### II. Assay Procedure (Batchwise)

1. Pipet a known aliquot of the prepared enzyme into a buffered substrate solution. Keep the suspension well mixed during the reaction period.
2. After the appropriate interval of time, stop the reaction by removing the insoluble enzyme, either by filtering or by centrifuging.
3. Assay the clear supernatant fluid (or filtrate) for the extent of the reaction in the manner normally used for the soluble enzyme.

#### III. Assay Procedure (Columnwise)

1. Pour or pipet a known quantity of the prepared enzyme into a chromatographic tube. For enzyme preparations which are very active, it is sometimes desirable to slurry the insoluble enzyme with an inert diluent to increase the bed volume of the packed enzyme. (NOTE: For agarose enzymes, use Sephadex or agarose as diluent. For acrylic beads enzymes, use Oxirane acrylic beads or macrobeads (Sigma Product Nos. O 7628 or O 9754) as diluent. For polyacrylamide or carboxymethyl cellulose enzymes, use cellulose or Sigmacell as diluent.)

2. Drain off excess buffer, and pass through the column a bed volume or so of buffered reaction mixture which is lacking one of the substrates.
3. Discard the equilibrating effluent.
4. Pass the reaction mixture, complete with all of the essential chemicals, through the bed at a constant flow rate. If temperature, substrate concentration, and enzyme are kept constant, the amount of conversion will be a function of the flow rate.
5. Assay the effluent for the extent of reaction as in the batchwise procedure.

3. a. Wash the insoluble enzyme in a column (or batchwise with centrifuging or filtering) with 0.1 M borate buffer, pH 9.0, to remove the substrates.
- b. Drain off excess buffer
- c. Dry the insoluble enzyme in a vacuum desiccator.
- d. Store the dry powder at 2-8 °C until you are ready to re-use it. It retains activity for many months in this form.

#### **IV. Re-use of the Insoluble Enzyme**

1. Wash the insoluble enzyme with water and/or buffer until free of substrates. It is now ready for another re-use cycle. It may be stored in this form for 2-3 days at 2-8 °C.
2. If long term storage (more than 2-3 days) is desired, return the agarose enzymes to the suspension medium indicated on the label. For the polyacrylamide and carboxymethyl cellulose enzymes, it is best to convert them to a dry form as follows:

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