DVS Activated Beads

(Cat. 1007-5/10/25)

DVS Activated Sepharose gel **ver. 1.1, 29.12.04**

Introduction

These widely useful rigid beads contains high density of Divinyl Sulfone (DVS) reactive groups. This matrix is suitable for binding biomolecules such as proteins and carbohydrates that present amino, thiol, or hydroxyl groups. The DVS activated beads are stable for up to 12 months when stored refrigerated and light-protected. Coupling reactions may be done between pH values 6-10 pH.

DVS Activated Beads characteristics.

Matrix: Sepharose™ CL-4B
Activation method: Oxiran.
Binding capacity: ~2-4 mg pure per ml
Mean bead size: 60-110 µm
Bead structure: Highly cross-linked spherical agarose, 4%
Max back pressure: 0.3 MPa, 3 bar
Max. flow rates: 15 ml/min/cm2
Recommended flow rate: 8-12 ml/min/cm2
Storage: 4°C in DDW.

Buffers Required for Protein Conjugation (using 10ml or 10gr of DVS Activated Beads)

Please Scale up or scale down all values when conjugating more or less than 10 ml of beads

DVS Activated Beads: 10gr
Double distilled water (100ml).
Neutral conjugation buffer (50ml): Phosphate buffer selaine (PBS) pH 6-8 , .
Basic conjugation buffer (50ml): Sodium bicarbonate buffer (pH-8-10).

A. Recommended protocol

1. Bring DVS Activated Beads from refrigerator to bench. Suspend beads in solution and remove by pipette the desired amount of beads. Wash beads 3 times with double distilled H_2O (ddH_2O). Washing could be done either using a suitable filter funnel or by centrifugations/suspension cycles of 1 minute long each one, done at approximately xg 600-1000. Do not exceed xg 1000 as beads may be deformed.
2. Prepare the protein/ peptide/ carbohydrate (biomolecules solution) in one of the recommended conjugation buffer at 1-20mg/ml. Higher concentrations, between 5-20 mg/ml are preferred to concentrations between 1-5 mg/ml.
3. Add two volumes of biomolecules solution to one volume of washed DVS Activated Beads in polypropylene tube and mix gently.
4. Sample 100ul upper supernatant (without beads) as time zero reference sample and store in the refrigerator.
5. Mix slowly overnight at 4°C to 25°C (reaction is insensitive to temperature), preferably with the use of a rocker. **Do not use magnetic stirrer for mixing**
6. Sample 100ul upper supernatant (without beads) for conjugation efficiency determination.
7. Read both samples diluted to 0.1 to 0.5mg/ml protein using a spectrophotometer at OD 280.
8. Wash beads three times, 2 minutes each time with the conjugation buffer at room temperature, in order to remove unbound biomolecules.
9. Add approximately two gel volumes 0.1M ethanolamine or 0.1M Tris Base to block unconjugated DVS groups. Stir gently for 2 hours.
10. Wash gel three times 2 minutes each time, with 5 volumes of saline (0.7%w/v NaCl in ddH_2O) added with 0.05% azide w/v, at room temperature, in order to remove unbound ethanolamine or Tris.

C. Storage

Store gel refrigerated in any desired buffer added with 0.1% azide w/v until use.