# Gelatin Beads

Cat. 6025-5/10/25

Affinity purification beads

ver. 1.1 04.07

## Introduction

Gelatin affinity media is commonly used for the purification of various proteins, mainly serum proteins, by affinity chromatography. Adar's Gelatin Beads are made with highly purified Bovine Gelatin and capable of purifying human fibronectin.

## Gelatin Beads Specifications

Matrix: Sepharose$^{TM}$ CL-4B
Coupling method: TargetLock.
Gelatin density: 2-3 mg/ml of gel
Binding capacity: ~1 mg of Fibronectin
Mean bead size: 40 -165 µm
Bead structure: Highly cross-linked spherical agarose, 4%
Max back pressure: 0.3 MPa, 3 bar
Max. flow rates: 4 ml/min/cm$^2$
Recommended flow rate: 1-3 ml/min/cm$^2$
Storage: 4°C in PBS pH 7.4 added with $NaN_3$ 0.1% (w/v) as a preservative.

## Protocol: Affinity-purification of Fibronectin using Gelatin Beads

### A. Buffers required

Binding buffer (200ml): 50mM $K_2HPO_4$, 0.15N NaCl pH 7.4.
Wash buffer (200 ml): Same as binding buffer - 50mM $K_2HPO_4$, 0.15N NaCl pH 7.4.
Elution buffer (100ml): 50mM Sodium acetate, 1M NaBr pH 5.0.
Storage buffer: PBS with 0.1% sodium azide as a preservative.

### B. Affinity-purification

1. Pack affinity column such as 1cm inner diameter Bio-Rad Ecno Pac with 5-7 ml Gelatin beads.
2. Equilibrate column with 20 volumes of binding buffer.
3. Load 100-200ml plasma containing sodium citrate on column at 0.5-1.0 ml/min. at room temperature on at 4°C.
2. Wash column with 20 volumes of wash buffer.
3. Elute Fibronectin with Elution buffer.

### C. Re-equilibration and Storage

1. Wash column with 10 bed volumes of Binding buffer, immediately at the end of elution step.
2. Storage conditions: Store column in a refrigerator with Storage buffer.