

# Hi-Bind™ T-Gel-Agarose

Store at 4°C. Do not freeze.

09/17

Cat. No.	M1313-1	1 ml
	M1313-10	10 ml
	M1313-100	100 ml

- I. **CONTENTS:** Supplied as 50% slurry in 20% Ethanol/H<sub>2</sub>O.
- II. **STORAGE:** Store at 4°C. Do not freeze. Stable, as supplied, for at least 1 year.
- III. **BINDING CAPACITY:** Binding of IgG ≥ 30 mg human or rabbit IgG/ml Hi-Bind™ T-Gel Agarose.
- IV. **FLOW RATE TESTED\*:** 2.89 ml/min \*Test condition: Calculations based on the time required to pass 18 ml of water through 2 ml settled beads (column diameter 1.5 cm).
- V. **MAXIMUM FLOW RATE\*\*** = 1800 cm/hr  
\*\*The highest flow that beads withstand for 1 min, without collapsing and the pressure reaching 1 MPa.
- VI. **USAGE:** Reusable for up to 10 times without significant loss of binding capacity.

## VII. Introduction:

Thiophilic Adsorption Chromatography (TAC) is a group-specific, salt-dependent purification technique with distinct adsorption affinity towards immunoglobulins and α<sub>2</sub>-macroglobulins. The term “thiophilic” refers to an affinity for sulfone groups that lie in close proximity to thioether groups. TAC has proven to be an easy and economical method for purification of many types of proteins, especially immunoglobulins. What makes TAC attractive is the ability to purify all types of immunoglobulins under universal and mild conditions (neutral pH). BioVision's Hi-Bind™ T-Gel Agarose beads provide up to 30 mg protein per ml settled beads capacity and allow rapid purification of any type of immunoglobulins from serum, ascites, tissue culture including IgG, IgE, IgM, IgA, IgY and Fab fragments.

## VIII. Applications:

- Purification of monoclonal and polyclonal antibodies from culture media, serum, ascites fluid, hybridoma supernatants, egg yolk.
- HTP purification of antibody clones under universal mild conditions

## IX. User Supplied Reagents and Equipment:

- Loading buffer: 50 mM sodium phosphate; 0.5 M Na<sub>2</sub>SO<sub>4</sub> pH 7.2
- Sample buffer: 50 mM sodium phosphate; 1.0 M Na<sub>2</sub>SO<sub>4</sub> pH 7.2
- Elution buffer: 20 mM sodium phosphate; 20% Glycerol pH 7.2
- Disposable columns 5 ml/25 ml (BioVison's Cat. No. M1314-50/M1315-50)

## X. PROTOCOL:

Carefully pack the column avoiding air bubbles. This protocol is for purifying immunoglobulins from serum, ascites or tissue culture.

1. Equilibrate the column with 5X resin bed volume of Binding Buffer & allow the buffer to drain through the column. Do not let the resin bed dry.
2. Dilute serum sample with Sample Buffer (1:1 ratio).
3. Mix well the diluted serum sample. Make sure there are no bubbles in the sample solution.
4. Apply the diluted sample onto the column. Do not let the resin bed dry.
5. Collect the flow-through. Optional: Reapply the flow-through to the column & collect the sample. Repeat 4 times.
6. Wash the column 4 - 5 times with 5X volume of Loading Buffer.
7. Elute antibodies with Elution Buffer ~5X resin bed volume while collecting eluate in clean tubes.
8. Analyze protein concentration in eluted fractions by measuring the absorbance at 280 nm and combine the fractions with highest absorbance. 1 OD<sub>280</sub> = 0.73 mg/ml IgG.
9. To regenerate/store column: Wash with 5 volumes of 20% ethanol and store in the same solution at 4°C.

Figure A

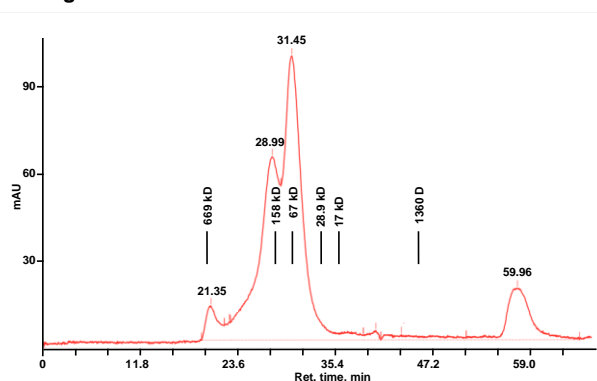


Figure B

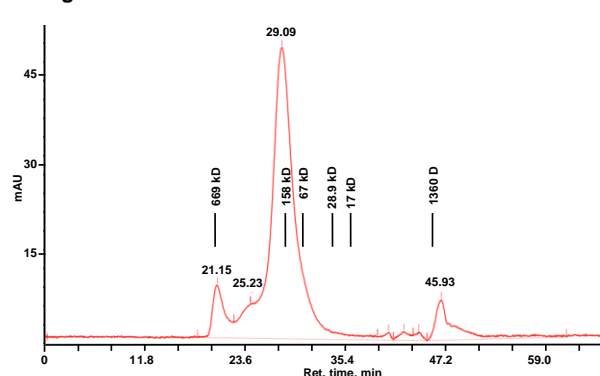


Figure: Analytical SEC of Human serum (Sample for TAC) – A and eluted IgG from Hi-Bind™ T-Gel Agarose – B on Superose 12 HR 10/30 column in 50 mM sodium phosphate; 0.3 M NaCl pH 7.2 at 0.4 ml/min.

**XI. Related Products:**

- Hi-Bind™ Protein A-Agarose (Cat. No. 6520)
- Hi-Bind™ Protein G-Agarose (Cat. No. 6513)
- Protein A-Agarose (Cat. No. 6526)
- Protein A-Sepharose (Cat. No. 6501)
- Protein A-Sepharose Column (Cat. No. 6508)
- Protein A-Magnetic Beads (Cat. No. 6507)
- Protein A Antibody (Cat. No. 5500)
- Protein A (Cat. No. 6500, 6500B)
- Protein A IgG Binding Buffer (Cat. No. 6524)
- IgG Elution Buffer (Cat. No. 6525)
- Protein A IgG Purification Buffer Kit (Cat. No. 6529)
- Hi-Bind™ Protein G-Agarose (Cat. No. 6513)
- Protein G-Sepharose (Cat. No. 6511)
- Protein G-Sepharose Column (Cat. No. 6518)
- Protein G-Magnetic Beads (Cat. No. 6517)
- Protein G (Cat. No. 6510)
- Protein G Antibody (Cat. No. 5510)
- Protein G-Biotin (Cat. No. 6215)
- Protein L-Sepharose (Cat. No. 6531)
- Protein L-Sepharose Column (Cat. No. 6538)
- Protein L Magnetic Beads (Cat. No. 6537)
- Protein L Antibody (Cat. No. 5530)
- Protein L (Cat. No. 6530)
- Protein A/G-Sepharose (Cat. No. 6503)
- Protein A/G-Sepharose Column (Cat. No. 6528)
- Protein A/G Magnetic Beads (Cat. No. 6527)
- Protein A/G (Cat. No. 6502)
- Protein A/G/L-Sepharose (Cat. No. 6541)
- Protein A/G/L-Sepharose Column (Cat. No. 6548)
- Protein A/G/L Magnetic Beads (Cat. No. 6547)
- Protein A/G/L (Cat. No. 6540)
- Protein G Coated 96-well Plate (Cat. No. 6522)

***FOR RESEARCH USE ONLY! Not to be used on humans.***