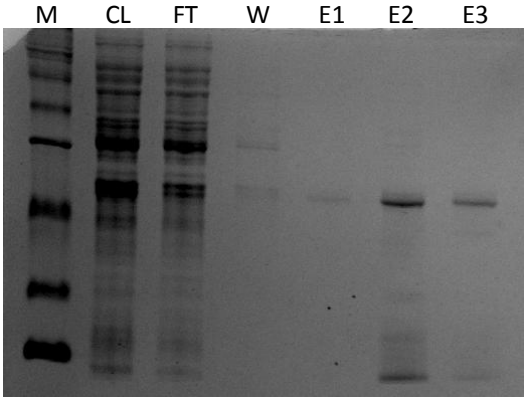
BioBharati LifeScience Pvt. Ltd.
Developing Best Products for Quality Research of National Significance
EN-35, Salt Lake, Sector-V, Kolkata, INDIA, PIN: 700091
E-mail: biobharati@gmail.com,Web: www.biobharati.com
Mobile No.: +91-9836508080, Land Line: +91-33-40077640.

Anti-GFP IgG Agarose Beads

Cat # BBGF 002

The green-fluorescent protein (GFP) (27 KDa) functions as a bioluminescence energy transfer acceptor in the Jellyfish Aequorea that maximally absorbs light at 395 nm and has an emission spectrum that peaks at 509 nm. Anti-GFP antibody is the tool for detection of GFP-tag proteins by different immunological techniques. This antibody is produced in rabbit by repeated injections of Immunogen.



SDS gel (12.5%) profile of purification of green fluorescence protein from crude bacterial cell lysate using anti-GFP IgG Agarose bead. M: mw marker; CL: crude lysate; FT: flow through; W: wash; E1,E2,E3: elution 1,2,3

Anti-GFP IgG was covalently coupled to solid support Agarose beads to prepare affinity purification matrix to purify GFP or GFP tagged proteins. Approximately 2 mg of anti-GFP antibody has been coupled with Agarose beads and the affinity matrix has purification capacity of ~1 mg free green fluorescence protein from crude bacterial cell

TECHNICAL SPECIFICATIONS

BEAD GEOMETRY & SIZE	: Spherical, ~ 50 - 150 µm diameter
CROSSLINKED	: Yes
BEAD AGAROSE %	: 6%
ACTIVATING GROUP	: Carbonyl
COUPLED ANTIBODY	: ~2 mg antibody/ml pack bead
PURIFICATION CAPACITY	: ~1 mg GFP protein/ml pack bead
MATRIX STABILITY	: Stable in all commonly used reagents
STORAGE SOLUTION	: 20% aqueous ethanol
STORAGE TEMPERATURE	: 4°C to 8°C. **DO NOT FREEZE.**

Continued from previous page…..

Protocol for affinity purification

Carry all procedure at 4°C, unless specified (cold room). Bacterial/Mammalian cell pellet containing the desired protein (GFP/GFP-tagged protein) was resuspended in 0.1M Phosphate buffer, pH 7.3 containing 0.15M NaCl and lysate was prepared by sonication/French press or any other method. Centrifuge the lysate at 13,500 rpm for 30 mins at 4°C to pellet the cellular debris. Collect the clear supernatant for purification process.

- Wash the bead with sufficient water to remove the preservative solution completely (select the bead volume depending on the approximate protein amount to be purified).
- Wash the bead with desired buffer (generally 0.1M PBS pH 7.3) and equilibrate in it for 15-30 minutes.
- Incubate the protein mixture (cell lysate) containing the desired protein to be purified with the bead for 1-2 hrs in 4°C, under gentle shaking.
  [For better purification carry the binding in dilute condition]
- Load mixture in gravity flow column and collect the pass through as unbound fraction (use BioBharati Empty Gravity Column BB-EGC06/012/030).
- Wash the bead with approx 20-25 bead volume of suitable buffer (PBS) at 4°C with occasional resuspension of the bead.
- Make the elution with 100 mM Glycine-HCl pH 2.8 containing 0.1% tween-20.
  [Make the elution stepwise in small fractions every time.
  Resuspend beads every time after addition of elution buffer and allow for few minutes.]
- Collect every fraction in 2 ml micro-centrifuge tube containing 80 µl Tris-HCl pH 8.8 and 200 µl 3M KCl for total 2 ml of final collected volume after elution.
- Choose right fraction containing high amount of desired protein/Ab by protein estimation and monitoring through SDS PAGE.
- Concentrate the fractions, dialyze it in desired buffer and store in -20°C/4°C according to its temp sensitivity.

For long term use and to keep the purification performance of bead good, it is important to wash the bead properly every time after purification by flushing plenty of double distilled water thoroughly with repeated resuspension and store it in 20% aqueous ethanol at 4°C.

[For anymore technical assistance please communicate our R&D section]