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**Anti-GFP\_Affinity beads Technical Manual**

[**Anti-GFP\_Affinity beads** **Technical Manual** 0](#_Toc154493426)

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For laboratory or further manufacturing use only

1. Description

GFP (Green Fluorescent Protein) or its mutants EGFP (Enhanced Green Fluorescent Protein) is widely used to detect the efficiency of gene expression as well as the expression and distribution of target proteins. eGFP as a tag protein, its fusion target protein self-fluorescence, without the need for antibodies to the target gene or hybridization to know the localization of the target gene in the cell, other substances with little interference. Anti-GFP\_Affinity beads(Biortus) is characterized by high affinity, high specificity, high sensitivity and stable nature, and is mainly used for affinity purification and detection of GFP and eGFP-tagged fusion proteins containing GFP and eGFP tagged fusion proteins in the commonly used protein expression systems such as prokaryotic and eukaryotic cells.

Table 1: Basic characteristics of the product

|  |  |
| --- | --- |
| Product content | 50% Anti-GFP\_Affinity beads in phosphate buffer containing 0.1% ProClean 950 |
| Use | Affinity purification |
| Matrix | 4% highly cross-linked agarose |
| Average bead size | 45-165 μm |
| Radicals | Anti-GFP Nanobody |
| Binding capacity | ~3 mg eGFP（~26 kDa）/ml Anti-GFP\_Affinity beads |
| Storage and stability | 2~8°C, **Avoid frozen packing at or below 0 ° C.** |

2. Instruction for Use

Considering the complexity of the samples and the diversity of experimental conditions, the following experimental instructions are for reference only, and can be adjusted according to the actual situation during the operation to obtain better experimental results.

**2.1** Equipments and Reagents Required but not Supplied

Micropipettors, Microcentrifuge tubes, Vortex mixer, centrifuge, Empty Columns, Protease inhibitor reagents, Buffers (Table 2 lists)。

Table 2. Buffer solutions required for empty column chromatography

|  |  |  |
| --- | --- | --- |
| **Purpose** | **Reagent abbreviation** | **Suggested** |
| Lysis buffer | HEPES | 20 mM HEPES, 150 mM NaCl, pH 7.5 |
| Commercialization | N/A |
| Balanced buffer | HEPES | 20 mM HEPES, 150 mM NaCl, pH 7.5 |
| Wash buffer |
| Storage buffer | PBS | 10 mM NaPhosphate, 200 mM NaCl, pH 7.0, 0.1% ProClean 950 |

**2.2** Sample Preparation

Depending on the nature of the sample, treatment is carried out to obtain a supernatant.

**2.3** Binding Procedures

The supernatant was incubated with equilibrated packing or flow-through with a preloaded column.

**2.4 Wash**

Rinse the packing with 25 CV of HEPES wash buffer in the column after collecting the flow-through solution. Take care to keep the packing moist.

**2.5 Digestion on beads**

At the end of the wash, according to the enzyme cleavage site of the fusion protein, the corresponding enzyme was added to the centrifuge tube for column cleavage, and the centrifuge tube was placed in an incubation spinner and spun for cleavage at 4°C overnight. Or cyclic digestion in preloaded columns.

**2.6 Flow through**

At the end of the incubation, collect the flow-through solution into a clean centrifuge tube, and continue to add 3CV wash buffer to continue to collect into the centrifuge tube after the end of the flow-through.

**2.7 Protease removal**

The collected flow-through solution was slowly flowed through the de-enzymatic packing material equilibrated with washing buffer to collect the target protein.

3. Reagents Compatibility Table

The tolerable concentration of listed reagents are tested by addition of these reagents at indicated  
concentrations.

Table 3. Reagents Compatibility

|  |  |  |
| --- | --- | --- |
|  | **Reagent** | **Maximum Tolerable concentration** |
| Detergent | DDM | 20% |
| LMNG | 20% |
| GDN | 20% |
| reducing agent | TCEP | 5 mM |
| β‐ME | 150 mM |
| DTT | 100 mM |
| Other | Glycerol | 50% |
| Salt | KCl | 1 M |
| NaCl | 1 M |

4. Troubleshooting

|  |  |  |
| --- | --- | --- |
| **Problem** | **Possible Cause** | **Solution** |
| Protein found in the flow through | Binding time is not enough | Increase the binding time experimentally |
| Failure to binding during the reaction | Adjustment of the reaction conditions during incubation of the sample with the filler so that the filler and the sample are well combined |
| Column is overloaded | Reduce the amount of the sample added to the resin or increase the amount of resin. |
| Reagent compatibility problem | Refer to the Reagent Compatibility Table to determine if there is too much reagent in the sample. If there is excess, perform Buffer replacement on the sample prior to purification. |
| No tagged protein exists in the eluate. | Unsuccessful of digestion | Ensure that active enzymes are used. Increase digestion time |
| Very low protein expression level | Optimize expression conditions to raise the protein  expression level. |
| The target protein has been  degraded | Use freshly prepared sample.  Perform purification at lower temperature, such as 4 °C.  Include protease inhibitors to the sample during cell lysis and binding steps. |
| **Multiple protein**  **bands found in the**  **eluate.** | Insufficient washing | Ensure adequate suspension and increase the number of washes, incubating each wash for 5-10 min.  Increase the concentration of salt ions in the wash solution and use super nuclease to remove nucleic acid effects. |