GST-Tag Elution Buffer (with Glycerol)

Introduction

GST-Tag Elution Buffer (with Glycerol) elutes the tagged protein from the beads by competing the reduced GSH with glutathione on the beads to bind the fusion protein. This product contains glycerol, which not only reduces the polarity of the target protein, but also protects the activity of the target protein. For eluents that require TritonX-100 and glycerol, GST-Tag Elution Buffer (with Triton and Glycerol) (K4202) can be used.

Protocol

1. The cell lysate was centrifuged at high speed, the supernatant was added to the prepared Beads, and incubated on a rotaster for at least 1 h at 4°C with rotation.
2. After transient centrifugation, retain some of the supernatant, test the media binding capacity with SDS-PAGE electrophoresis, and leave the beads.
3. Add 1 mL of GST wash solution per 0.5 mL of beads to wash, and leave the beads after instantaneous centrifugation. Repeat 3 times.
4. 0.6 mL of GST-Tag Elution Buffer (with Triton and Glycerol) was added to every 0.5 mL beads and incubated for 1 h at 4°C with gentle rotation on a rotating instrument.
5. Transiently centrifuged the precipitated beads, retained the supernatant (equivalent to the eluent), prepared samples, and detected the eluted proteins by SDS-PAGE electrophoresis.

***Note:**

1. The binding of GST fusion protein to reduced glutathione is relatively slow, and sufficient action time needs to be ensured in order to obtain the maximum binding amount. The binding efficiency of different GST fusion proteins was significantly different.
2. The reduced glutathione concentration, elution volume, and elution time required for optimal purification of GST fusion proteins may vary. SDS-PAGE and Western hybridization analysis of the flow-through and eluate is necessary to determine the optimal purification conditions.
3. The GST Assay Kit assists in optimizing elution conditions and analyzes each step of the purification process, and the kit focuses on the detection of GST-tagged proteins with biochemical or immunoassays.
4. The absorption value at A280 can be used to estimate the GST-tagged protein concentration, which corresponds to a concentration of 0.5 mg/mL for about A280~1.
5. GST-tagged protein concentrations can also be determined using standard chromogenic methods (e.g., Lowry, BCA, and Bradford methods). If the Lowry and BCA methods are used, the sample is first desalted or dialyzed in PBS to remove glutathione, as glutathione interferes with protein absorption, while the Bradford method is not affected by glutathione.

6. The reuse of the medium, depending on the nature of the sample, must be the same sample to prevent cross-contamination.

Note

1. Storage conditions: Store at 4°C for half a year.
2. Protein degradation is relatively easy, so be sure to do each step on ice, and store at 4°C or -20°C after collecting the protein of interest.
3. This product is for scientific research use only.

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