Coomassie (Braford) Staining Solution （for Protein Determination）

C1492574

Storage 2-8°C .

Introduction

This product is a ready-to-use protein quantification reagent based on the Bradford method, with Coomassie Brilliant Blue G-250 as its active component. Upon binding to proteins, the dye shifts to a blue form and exhibits strong absorbance at 595 nm. The change in absorbance is proportional to the protein concentration, enabling accurate quantification.

Instructions for Use

The specific method of use may vary depending on the purification tool employed.

1 Sample Preparation

Before using the Protein A/G purification column, serum or ascites samples must be clarified by centrifugation or filtration through a 0.45 μm filter. If hybridoma culture supernatant appears clear to the naked eye, it can be used directly; otherwise, it should be clarified by centrifugation or filtration.

2 Sample Purification

Note: The purification column is designed to be used in a vertical position during loading, washing, and elution steps. Care should be taken to avoid introducing air bubbles into the column, as they can reduce separation efficiency and increase the pressure required to achieve adequate flow rates. Bubbles can be removed by gently tapping the column.

2.1 Column Preparation

2.1.1 Remove the column stored at 4°C and drain the 20% Ethanol from the column.

2.1.2 Fill the column with 1× Binding Buffer (diluted from 10×) (P1492570) to equilibrate it (approximately 15 column volumes). Wash the column by allowing the buffer to flow through at a rate of approximately 1 mL/minute.

2.2 Loading

Fill a 10 mL syringe with the sample. Load the sample onto the Protein A/G column by allowing the sample-binding buffer mixture to flow through the column at a rate of approximately 0.5 mL/minute.

2.3 Equilibration

Fill the column with 1× Binding Buffer (diluted from 10×) (P1492570) for equilibration (approximately 15 column volumes). Equilibrate at a flow rate of approximately 1 mL/minute.

2.4 Elution

2.4.1 Add 3 ml (3 column volumes) of 1× Elution Buffer (diluted from 5×)（P1492569） per column. Collect the eluate by gravity. Prepare several 1.5 ml centrifuge tubes in advance, each containing 10 μl of Neutralizing Buffer (P1452572) for neutralizing the eluate.

2.4.2 When collecting the eluate, add approximately 8 drops of eluate into each 1.5 ml tube, and so on. Mix well and monitor the protein concentration change using G250（C1492574）.

2.5 Equilibration

Fill the column post-elution with 1× Binding Buffer (diluted from 10×) (P1492570) for equilibration (approximately 15 column volumes). Equilibrate at a flow rate of approximately 1 mL/minute.

2.6 Column Storage

2.6.1 After antibody elution is complete, continue by washing the column with 10 mL of Elution Buffer（P1492569） to avoid antibody residue.

2.6.2 Wash the column with 20 mL of Binding Buffer to wash and equilibrate the resin.

2.6.3 Wash the column with 15 mL of 20% Ethanol, then add 5 mL of 20% Ethanol and store the column at 2-8°C.

2.7 Regeneration

2.7.1 Wash the column with 20 mL of Regeneration Buffer（P1492573）.

2.7.2 Rinse the column with 30 mL of ddH_2O to thoroughly remove the regeneration buffer.

2.7.3 Wash the column with 20 mL of Binding Buffer to wash and equilibrate the resin.

2.7.4 Wash the column with 15 mL of 20% Ethanol, then add 5 mL of 20% Ethanol and store the column at 2-8°C.