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## Protein A/G Binding Buffer (10x)

**P1492570**

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**Storage** 2-8°C

### Introduction

This product is a binding buffer specifically designed for the purification of antibodies (IgG) using Protein A/G affinity chromatography media. Its optimal pH and ionic strength ensure specific, high-affinity binding of IgG antibodies from various species (such as human, rabbit, mouse, rat, etc.) to the Protein A/G media, while minimizing non-specific binding of impurities. This provides an ideal environment for obtaining antibodies with high purity and yield.

### Instructions for Use

Prepare 1X Binding Buffer by diluting Binding Buffer(10X) with deionized water. 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  $\mu$ m 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 1X Binding Buffer (diluted from 10X) 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 1X Binding Buffer (diluted from 10X) 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 1X Elution Buffer (diluted from 5X) (P1492569) per column. Collect the eluate by gravity. Prepare several 1.5 ml centrifuge tubes in advance, each containing 10  $\mu$ l of Neutralization Buffer (P1492572) 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 (P1492574) .

#### 2.5 Equilibration

Fill the column post-elution with 1X Binding Buffer (diluted from 10X) 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<sub>2</sub>O 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.