Temperature Responsive Protein A

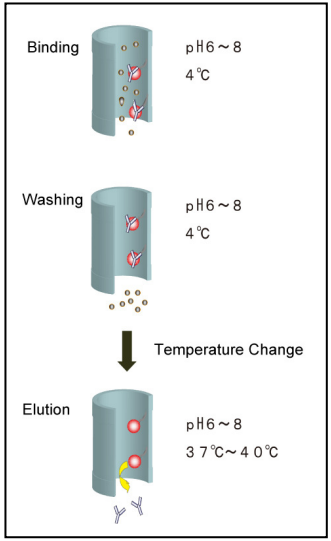
Byzen Pro®

Instructions

Nomadic Bioscience Co., Ltd.
ORIC 5303 Haga Kita-ku Okayama Japan 701-1221
Phone: +81(0)86-286-9507 Fax : +81(0)86-286-9508
Email : info@nomadicbio.com
URL: www.nomadicbio.com

1. DESCRIPTION OF THE PRODUCT

Byzen Pro® provides a new antibody purification scheme where antibody is eluted under neutral pH by changing temperature. With the conventional Protein A, antibody is eluted under strongly acidic conditions where antibody may be at risk of aggregation. In contrast, **Byzen Pro®** uses a neutral pH buffer for elution, avoiding risk of aggregation.



1. Antibody is eluted under neutral pH.
Byzen Pro® needs no acidic conditions. No risk of aggregation of antibody.

2. Binding and elution are controlled by temperature.
Antibody is bound below 10° C and eluted between 37 to 40℃.

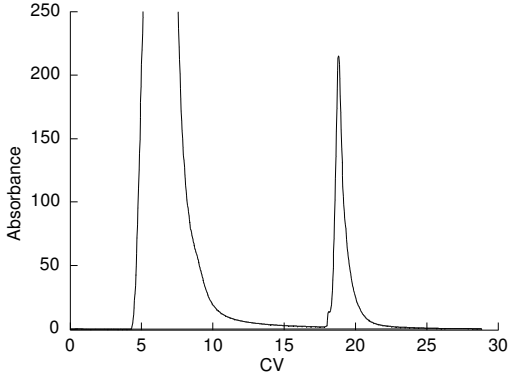
3. A buffer of your choice can be used for elution.
PBS buffer can be used for the Protein A step. Buffer exchange won't be necessary.

4. Good antibody specificity
Byzen Pro® is as specific as the conventional Protein A, showing excellent impurity elimination capability.

5. A recovery ratio is 95% or more.

2.1 PURIFICATION WITH A CLOSED COLUMN

CAUTION: Column pressure should stay below 0.3 MPa when operating chromatography equipment or a pump.



- Column Volume: 1.0 ml
- Bed Height: 2.5 cm
- Buffer: 20 mM Phosphate, 150 mM NaCl, pH 8.0
- Flow Rate: 1.0 ml/min
- Binding temperature: 4°C
- Elution temperature: 40°C
- Sample: Human IgG (1 mg) + cell lysate (1 ml)

PROCEDURE

1) Attach a 5 mL loop before the column, and wash the column with 5 CV of H_2O.
2) Immerse the column and the loop into a 4°C water bath and then equilibrate the column with buffer.
3) Wait for 5 min for temperature equilibration, and then inject a sample.
4) After wash the column to remove impurities, stop the pump. Take the column and the loop out of the 4°C water bath and immerse them into a 40 °C water bath.
5) Wait for 5 min for temperature equilibration, and then start the pump to elute antibody.

REGENERATION

Note: Reuse of Byzen Pro® depends on conditions. The follow procedure represents merely an example.

7) Bring the column to room temperature, and wash the column with 5 CV of H_2O.
8) Wash the column with 5 CV of 6 M guanidinium or 8 M urea (This step must be performed at room temperature to prevent precipitation of guanidinium or urea. Care should be taken not to exceed the max pressure).
9) Wash the column with more than 10 CV of H_2O. Then repeat the purification from 2).

2.2 PURIFICATION WITH OPEN COLUMN HAVING JACKET

We recommend the use of a column with a jacket to control temperature with circulating water when using an open column. It is also recommended to calibrate a time required to equilibrate the column before use.

When a flow rate by gravity is too slow, use an aspirator, a pump or a syringe to withdraw buffer from the column.

・Buffer 20 mM Phosphate, 150 mM NaCl, pH 8.0
・Flow Rate: Gravity
・Binding temperature: 4°C
・Elution temperature: 40°C

PROCEDURE

1) Pack the column with **Byzen Pro®** using a pipette (a bed height of 2 cm or more is recommended when the concentration of antibody is low).
2) Set the temperature of the column to be 4°C with circulating water, and then run the column with 5 CV of buffer by gravity (buffer should be re-cooled at 4°C).
3) Make sure that the column temperature is 4°C, and gently apply a sample onto the surface of the resin to allow the sample to flow by gravity.
4) Apply a 4°C buffer onto the surface of the resin without disturbing the surface to wash the column. Repeat this step until all impurities are removed.
5) Change the temperature of the circulating water to 4°C, and wait until equilibrated.
6) Apply a 40°C buffer onto the surface of the resin without disturbing the surface to elute antibody by gravity.

REGENERATION

Note: Reuse of Byzen Pro® depends on conditions. The follow procedure represents merely an example.

8) Bring the column to room temperature, and wash the column with 5 CV of H_2O.
9) Wash the column with 5 CV of 6 M guanidinium or 8 M urea (This step must be performed at room temperature to prevent precipitation of guanidinium or urea. Care should be taken not to exceed the max pressure).
10) Wash the column with more than 10 CV of H_2O. Then repeat the purification from 2).

2.3 PURIFICATION WITH OPEN COLUMN (NO JACKET)

Purification may be performed by placing the column in a cold room/refrigerator or an incubator set at desired temperature. It is recommended to calibrate a time required to equilibrate the column before use.

When a flow rate by gravity is too slow, use an aspirator, a pump or a syringe to withdraw buffer from the column.

・Buffer 20 mM Phosphate, 150 mM NaCl, pH 8.0
・Flow Rate: Gravity
・Binding temperature: 4°C
・Elution temperature: 40°C

PROCEDURE

1) Pack the column with **Byzen Pro®** using a pipette (a bed height of 2 cm or more is recommended when the concentration of antibody is low).
2) Steps 3) and 4) should be performed in a cold room or by placing the column in a refrigerator set at 4°C.
3) Equilibrate the column with 5 CV of buffer by gravity (buffer should be pre-cooled at 4°C).
4) Make sure that the column temperature is 4°C, and gently apply a sample onto the surface of the resin to allow the sample to flow by gravity.
5) Apply a 4°C buffer onto the surface of the resin without disturbing the surface to wash the column. Repeat this step until all impurities

are removed.

6) Steps 7) and 8) should be performed by placing the column in an incubator set at 40°C.

7) Make sure that the column temperature is 40°C, and gently apply a 40°C buffer onto the surface of the resin without disturbing the surface to elute antibody by gravity.

REGENERATION

Note: Reuse of Byzen Pro® depends on conditions. The follow procedure represents merely an example.

9) Bring the column to room temperature, and wash the column with 5 CV of H_2O.

10) Wash the column with 5 CV of 6 M guanidinium or 8 M urea (This step must be performed at room temperature to prevent precipitation of guanidinium or urea. Care should be taken not to exceed the max pressure).

11) Wash the column with more than 10 CV of H_2O. Then repeat the purification from 2).

2.4 STORAGE

1) Wash the column with 5 CV of H_2O.

2) For a closed column, replace water with 20% ethanol and store at 4°C.

3) For an open column, replace water with 20% ethanol. Take the resin out of the column and store in a closed container at 4°C.

3. SPECIFICATION

Bead Structure	Cross-linked polyvinyl alcohol
Mean Particle Size	70 μm
Max. Back Pressure	0.3 Mpa、3 bar
Flow Rate (Recommend)	125 to 250 cm/h
pH Range (Recommend)	pH 6 to pH 8
Binding Temperature	4°C
Eluting Temperature	37. to 40°C
Dynamic Binding Capacity	26 mg/ml (250 cm/h)
(20 cm column)	34 mg/ml (125 cm/h)
Storage Condition	20% ethanol at 4°C

Specificity
Human IgG_1, IgG_2 and IgG_4
Rabbit IgG and Goat IgG

4. CATALOG NUMBER

0809N05	5 ml
0809N25	25 ml

v 2.00