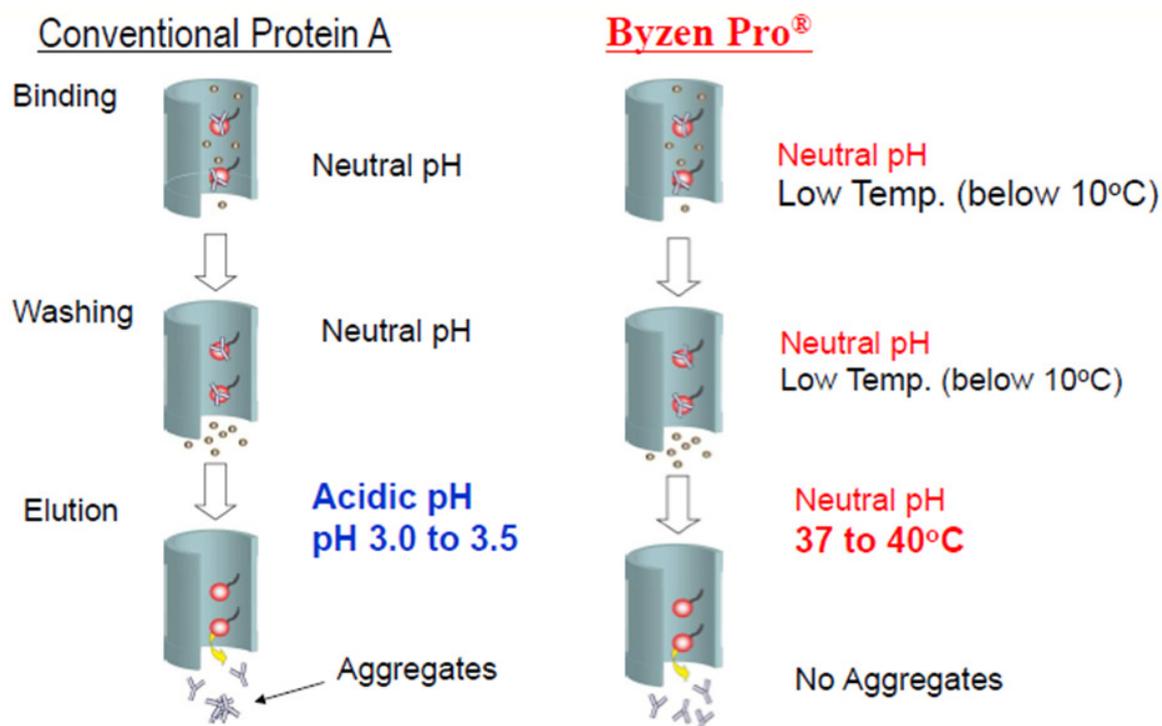


Product Information

Manual for Antibody Purification by Affinity Chromatography using 18887 Byzen Pro™ Protein A resin

This manual describes how efficiency in antibody downstream processing can be substantially improved leading to decreased manufacturing costs for therapeutic antibodies by eliminating the risks of antibody aggregation and degradation¹⁻⁶. The improvement results from a new elution principle whereby elution by strongly acidic buffers used in conventional protein A affinity chromatography are replaced by elution at neutral pH from the Byzen Pro™ Protein A resin by simply changing the temperature, which significantly increases the antibody recovery rate. The large knowhow about the structure and folding of protein A has led to engineered protein A variants with improved properties with regard to binding properties⁷⁻⁹.

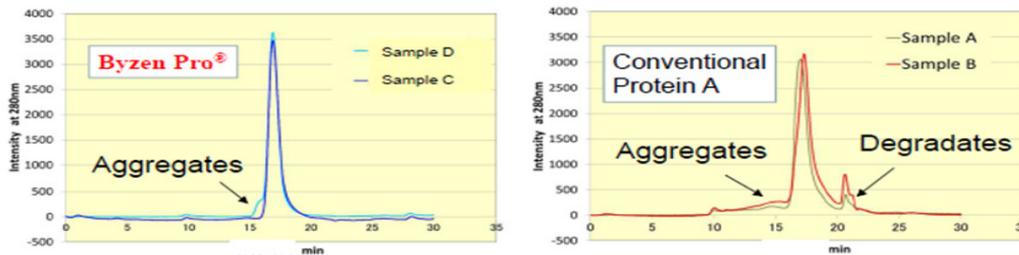


1. Product Description

The Byzen Pro™ Protein A resin (Product number 18887) consists of an engineered protein A immobilized on a cross-linked polyvinyl alcohol bead structure and provides a new antibody purification procedure¹ which allows to elute antibodies under neutral pH by simply changing temperature from low temperature to 37-40° C. Working at neutral pH avoids the risk of antibody aggregation. The mean particle size of Byzen Pro™ Protein A resin is 70 µm and the maximum back pressure 0.3 Mpa/3 bar. A flow Rate of 125 to 250 cm/h and a pH range of pH 6 to pH 8 is recommended. The new elution principle is based on a binding temperature of 4°C and an eluting temperature between 37°C and 40°C. This results for a 20cm column in a dynamic antibody binding capacity of 26 mg/ml (at a flow rate of 250 cm/h) and 34 mg/ml (at a flow rate of 125 cm/h). It is recommended to store the resin in 20% ethanol at 4°C.

- The antibody is eluted under neutral pH and Byzen Pro™ Protein A resin needs no acidic conditions. This minimizes the risks of antibody aggregation and antibody degradation.

Antibody purified with Byzen Pro® shows Less aggregation



Size exclusion chromatography

- Binding and elution are controlled by temperature. The antibody is bound below 10°C and eluted between 37°C to 40°C.
- A buffer of your choice can be used for elution. PBS buffer can be used for the Protein A step. Buffer exchange will not be necessary.
- Good antibody specificity. Byzen Pro™ Protein A resin is as specific as conventional Protein A resins, showing excellent capabilities for the elimination of impurities.

Species	Subclass	Conventional Protein A	Byzen Pro
Mouse	IgG ₁	++	-
	IgG ₂	++	-
Goat		++++	+++
Rabbit		+++++	+++++
Human	IgG ₁	+++++	+++++
	IgG ₂	+++++	++++
	IgG ₄	++++	+++++

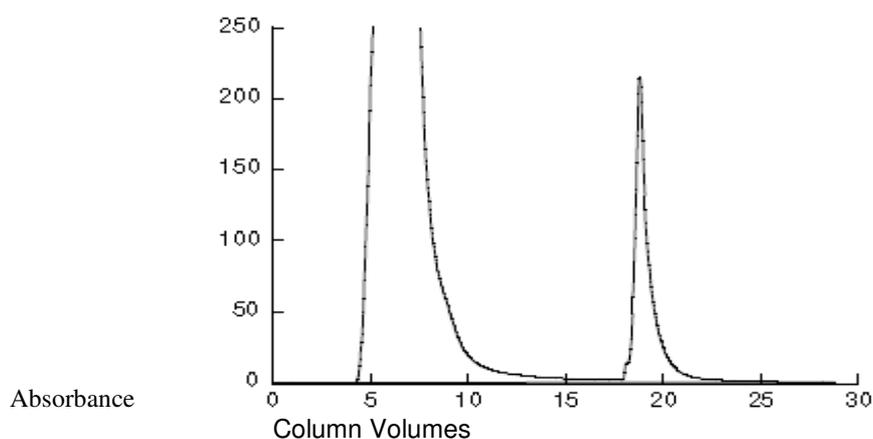
- Depending on the monoclonal antibodies and their stabilities at low pH, a higher recovery, in a specific case up to a 3-fold increase, can be achieved with the Byzen Pro™ Protein A resin compared to a conventional protein A affinity chromatography can be achieved.

2. Purification Procedures

Depending on the type of chromatography columns used, procedures for the antibody purification with a closed column, for the purification with a closed column having a jacket and for the purification with a closed column having no jacket are described in the following sections 2.1 to 2.3 .

2.1 Purification Procedure with a closed column

- 1) Connect a 5 mL loop to the top of the column, and wash the column with 5 column volumes (CVs) of H₂O.
- 2) Immerse the column and the loop into a 4°C water bath and then equilibrate the column with buffer.
- 3) Allow 5 min for temperature equilibration, and then inject a sample, and initiate the flow of buffer through the column.
- 4) After removing impurities, stop the pump. Remove the column and the loop from the 4°C water bath and completely immerse them into a 40°C water bath.
- 5) Wait 5 min for temperature equilibration, and then start the pump to elute antibody.



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)

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

6) Regeneration Note: The reuse of Byzen Pro™ Protein A resin depends on specific conditions. The following procedure represents an example only.

- 7) Bring the column to room temperature, and wash the column with 5 CVs of H₂O.
- 8) Wash the column with 5 CVs of 6 M guanidinium or 8 M urea (This step must be performed at room temperature to prevent precipitation of guanidinium or urea. A guanidinium or urea solution is very viscous, and may significantly increase column pressure. Care must be taken to prevent the column pressure from exceeding the defined limit.).
- 9) Wash the column with > 10 CVs of H₂O. Then repeat the purification procedures starting with step 2).

2.2 Purification Procedure with an open Jacketed column

An open jacketed column should be used for controlling temperature with circulating water. An advanced estimation of the time required to equilibrate the column is also recommended. If the gravity flow rate is too slow, an aspirator, pump or syringe can be used to aspirate buffer from the column. A buffer consisting of 20 mM Phosphate, 150 mM NaCl, pH 8.0 may be used. As before, the binding temperature is 4°C, and the elution temperature is 40°C.

- 1) Fill the column with Byzen Pro™ Protein A resin using a pipette (a bed height of ≥ 2 cm is recommended when purifying a low concentration of antibody).
- 2) Establish a column temperature of 4°C as regulated by circulating water. Then wash the column with 5 CVs of buffer by gravity (pre-cool the buffer at 4°C).
- 3) Make sure that the column temperature is 4°C, and then gently apply the sample onto the surface of the resin, and allow the sample to flow by gravity.
- 4) Wash the column by applying a 4°C buffer onto the surface of the resin without disturbing the surface, and let it flow through the column. Repeat this step until all impurities are removed.
- 5) Change the temperature of circulating water to 40°C, and wait until the temperature has equilibrated.
- 6) Apply a 40°C buffer onto the surface of the resin without disturbing the surface, and elute the antibody by gravity.
- 7) Regeneration Note: The reuse of Byzen Pro™ Protein A resin depends on specific conditions. The following procedure represents an example only.
- 8) Bring the column to room temperature, and wash the column with 5 CVs of H₂O.
- 9) Wash the column with 5 CVs of 6 M guanidinium or 8 M urea (This step must be performed at room temperature to prevent precipitation of guanidinium or urea. A guanidinium or urea solution is very viscous, and may significantly increase column pressure. Care must be taken to prevent the column pressure from exceeding the defined limit).
- 10) Wash the column with > 10 CVs of H₂O. Then repeat the purification procedures starting with step 2).

2.3 Purification Procedure with an open column having no jacket

Purification may be performed by placing the column in a cold room/refrigerator or an incubator set at the desired temperature. An advanced estimation of the time required to equilibrate the column is also recommended. If the gravity flow rate is too slow, an aspirator, pump or syringe can be used to aspirate buffer from the column. A buffer consisting of 20 mM Phosphate, 150 mM NaCl, pH 8.0 may be used. As before, the binding temperature is 4°C, and the elution temperature is 40°C.

- 1) Fill the column with Byzen Pro™ Protein A resin using a pipette (a bed height of ≥ 2 cm is recommended when purifying a low concentration of antibody).
- 2) Place the column in a cold room or a refrigerator set at 4°C.
- 3) Wash the column with 5 CVs of buffer by gravity (Pre-cool the buffer to 4°C).
- 4) Make sure that the column temperature is 4°C, and then gently apply the sample onto the surface of the resin, and allow the sample to flow by gravity.
- 5) Wash the column by applying a 4°C buffer onto the surface of the resin without disturbing the surface, and let it flow through the column. Repeat this step until all impurities are removed.
- 6) Place the column in a 40°C water bath.
- 7) Make sure that the column temperature is 40°C, and then gently apply a 40°C buffer onto the surface of the resin without disturbing the surface to elute antibody by gravity.

8) Regeneration Note: The reuse of Byzen Pro™ Protein A resin depends on specific conditions. The following procedure represents an example only.

9) Bring the column to room temperature, and wash the column with 5 CVs of H₂O.

10) Wash the column with 5 CVs of 6 M guanidinium or 8 M urea (A guanidinium or urea solution is very viscous, and may significantly increase column pressure. Care must be taken to prevent the column pressure from exceeding the defined limit).

11) Wash the column with > 10 CVs of H₂O. Then repeat the purification procedures starting from Step 2).

3. Storage

1) Wash the column with 5 CVs of H₂O.

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. Then removing the resin from the column, and store it in a closed container at 4 °C.

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The product 18887 Byzen Pro™ Protein A resin is routinely available in the three package sizes of 1ml, 5ml and 25 ml. The corresponding product numbers are 18887-1ml, 1887-5ml and 18887-25ml. For larger and bulk package sizes please inquire.

Precautions and Disclaimer:

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

use. Additional terms and conditions may apply. Please see product information on the Sigma-Aldrich website at www.sigmaaldrich.com and/or on the reverse side of the invoice or packing slip.