INSTRUCTION MANUAL

SERVA IMAC HD Test Kit

Agarose for Affinity Purification of His-Tag Fusion Proteins

(Cat. No.42160, 42161)

SERVA Electrophoresis

Contents

Ver 09/13

1. SERVA IMAC HD Test Kit

1.1. General information

SERVA IMAC HD Agarose Test Kits are a fast and easy way to screen different IDA Agaroses with high binding capacity for optimal affinity purification of His-tagged fusion proteins.

1.2. Kit Components

Cat. No.	Component	Size
42160 42161	SERVA IDA Metal-Free HDAgarose Resin SERVA Ni-IDA HD Agarose Resin SERVA Zn-IDA HD Agarose Resin SERVA Co-IDA HD Agarose Resin	2 ml each
42161	additionally contains mini columns	40 pieces

1.3. Storage conditions

Store at +2 °C bis +8 °C (35 °F – 46 °F). Do not freeze.
If stored at the recommended temperature, the product will be suitable for use until: see label.

2. Affinity purification of soluble proteins

Please note that these resins are adapted to work mainly in native conditions.

2.1. Elimination of the preservatives

Remove the lower cap of the mini column. Place the column in a microcentrifuge tube. Shake the test kit containing resin and add 400 µl of the suspension to the column. Centrifuge* and discard the flow-through (preservative residue) collected in the tube.

***Note:** In all centrifugation steps carried out in this procedure, usually a mild centrifugation (1,000 – 1,500 rpm) is sufficient.

2.2. Equilibration of the column

Equilibrate the column with 1 ml binding buffer.

Binding buffer:
The typical binding buffer is 20 mM Na_2HPO_4 (SERVA Cat. No. 30200), 500 mM NaCl (SERVA Cat. No. 30183), 10 mM imidazol (SERVA Cat. No. 26081), pH 7.5. The choice of buffer depends on the particular properties of the protein as well as of the type of chelate used. The buffers used most frequently are acetate (50 mM) or phosphate (10 - 150 mM). The pH of binding buffer generally leads to neutrality (pH 7.0 -8.0), but can vary over the range 5.5 – 8.5. To avoid ionic interchange, add 0.15 – 0.5 M NaCl.

> **Important:** In some cases to increase the selectivity of the binding of target protein it is necessary to add to the binding buffer a small amount of imidazole (10 - 40 mM). To avoid affecting the O.D. 280 nm it is important to use a high purity imidazole, e.g. SERVA Cat. No. 26081. It is also important to avoid the presence of agents like EDTA or citrate at all times.

2.3. Sample application

Once the resin is equilibrated, the sample containing the fusion protein for purification is applied. In some cases a slight increase of contact time may facilitate binding. Discard the flow-through.

2.4. Washing of the resin

It will be washed with the binding buffer until O.D. 280 nm reaches the baseline level. Discard the flow-through.

2.5. Elution of the fusion protein

The elution of the protein can be done in different ways:

2.5.1. Addition of competitive ligand

Addition of competitive ligand (generally imidazole), allows the elution of the retained protein.

Standard elution buffer:
20 mM Na_2HPO_4 (SERVA Cat. No. 30200), 500 mM NaCl (SERVA Cat. No. 30183), 500 mM imidazol (SERVA Cat. No. 26081), pH 7.5.

In general, 500 mM imidazole is enough to elute the protein. Most proteins are eluted with concentrations around 250 mM. It is also possible to increase the imidazole concentration up to 2.0 M if necessary. Other reagents that can be used as competitive ligands are histidine (His) and ammonium chloride.

Note:
Generally, the subsequent elimination of imidazole is not necessary. But if it is, it may be done by dialysis, precipitation with ammonium sulfate or ultrafiltration.

2.5.2. Reduction of the pH

Reduction of pH (with or without gradient), also allows the elution of the desired protein (pH 3.0 – 4.0).

2.5.3. Addition of other chelating reagents

A more drastic method uses reagents like EDTA or EGTA (50 mM), which causes the elution of both the fusion protein and chelating metal.

Note: For most of the applications it is not necessary to eliminate the His-tag. However, this elimination is necessary for certain applications such as X-ray, crystallography or RMN, where the protein structure is to be determined later. For these purposes, a His-tag is usually spliced to the protein, at a protease cleavage site.

3. Affinity purification of proteins forming inclusion bodies

The recombinant proteins often form insoluble inclusion bodies. If it is need to be rendered soluble by purification under denaturing conditions, using for example urea or guanidine chloride at relevant stages, please find below data of the chemical compatibility of the agarose beads.

	Reagents	
Chemical stability	10 mM HCl 100 mM NaOH 20 % (v/v) Ethanol 100 mM Sodium acetate, pH 4.0	2 % (w/v) SDS 30 % (v/v) 2-Propanol 1 M NaOH 70 % (v/v) Acetic acid
Denaturing agents	8 M Urea	6 M Guanidine-HCl
Detergents	2 % (w/v) Triton® X-100 2 % (w/v) Tween® 20	1 % (w/v) CHAPS
Additives	2 M Imidazole 20 % (v/v) Ethanol + 50 % (w/v) Glycerol 100 mM Na_2SO_4 1.5 M NaCl	1 mM EDTA 1 mM EDTA + 10 mM $MgCl_2$ 60 mM Citrate 60 mM Citrate + 80 mM $MgCl_2$
Reducing agents	10 mM Glutathion, reduced 20 mM 2-Mercaptoethanol	5 mM Dithioerythritol (DTE) 5 mM Dithiothreitol (DTT)
Buffers	50 mM Na_2HPO_4, pH 7.5 100 mM Tris-HCl, pH 7.5 100 mM MOPS, pH7.5	100 mM Tris-Acetate, pH 7.5 100 mM HEPES, pH 7.5

Pre-treatment of the column/resin to remove weakly attached cations.

1. Wash the resin with 5 column volmes of distilled water.
2. Wash the resin with 5 column volumes of binding buffer (w/o reducing agents)
3. Wash the resin with 5 column volumes of elution buffer (w/o reducing agents)
4. Equilibrated with 10 column volumes of binding buffer (w/o reducing agents)

4. Troubleshooting

4.1. Sample application

Observation	Causes	Recommendation
High viscosity of the sample	DNA in the sample	DNase- or sonication treatment
	Steric hindrance of the substrate	Dilution of the sample Batch format purification
Highly diluted or concentrated sample	Highly diluted sample	Sample concentration prior to column application Batch format purification
	Highly concentrated sample	Dilution of the sample

4.2. Adsorption

Observation	Causes	Recommendation
Target protein not bound to the column	His-tag is not present or has been degraded	Use of protease inhibitors Purification performed at + 4 °C
	His-tag is not exposed (inaccessible)	Purification under denaturing conditions Add tag in other site-terminus, or both positions
	Inadequate binding conditions	Check buffer and pH; reduce imidazole concentration Check whether buffer components interact with the matrix or not
Target protein binds only partially to the column	Column capacity is exceeded.	Apply less protein Regeneration of the column
	Loss of chelating metal	Regeneration of the column Avoid use of reducing and chelating agents
	His-tag is not very well exposed.	Reduce flow rate Batch format purification

Observation	Causes	Recommendation
Target protein binds only partially to the column	Poor protein expression	Optimization of the expression
	Formation of inclusion bodies	Modification of the bacterial growth Purification under denaturing conditions
	Formation of channels within the column	Re-pack column
	Low binding capacity	Use cation with higher binding capacity

4.3. Elution

Observation	Causes	Recommendation
High amount of co-eluted proteins	Insufficient washing stage	Increase volume of washing buffer Add imidazole (5-10 mM)
	Inadequate adsorption conditions	Check pH Add NaCl to avoid unspecific interactions Addition of non-ionic detergents, ethylenglycol or glycerol Increase imidazole concentration in the binding buffer
	Column too large	Reduce resin quantity
	Low selectivity of the column	Test of SERVA IDA LD Agarose Imidazole concentration gradient

Observation	Causes	Recommendation
Target protein elutes poorly	Too smooth elution conditions	Increase imidazole concentration Reduce pH Elution at higher temperature, if possible
	Too strong interaction between protein and chelating metal	Elution with EDTA Elution at pH 4.0 and with imidazole Using another agarose resin Increase imidazole concentration to 1 M Reduce flow rate Elution under denaturing conditions
	Precipitation of fusion protein	Add detergents Incubate the column with elution buffer for 8 - 10 h and elute with elution buffer Batch format of binding and elution
Elution profile is not reproducible in different cycles of purification	Modification of the sample, e.g. His-tag degradation because of protease activity	Prepare fresh samples Add protease inhibitors Purification at +2 °C - +8 °C
	Precipitations of proteins and/or lipids	Regeneration of the resin
	Variation of pH and/or ionic forces	Prepare new buffers
	Loss of binding capacity	Regeneration of the resin

4.4. Changes in the resin

Observation	Causes	Recommendation
Loss of color	Chelating agents in the sample	Purification of the sample and regeneration of the resin
Change of color	Reducing agents in the sample	Purification of the sample and regeneration of the resin

5. Ordering information

Product	Cat. No.
SERVA IMAC LD Test Kit	42162.01
SERVA IMAC LD Test Kit plus columns	42163.01
SERVA IMAC Ni-IDA Test Kit	42164.01
SERVA IMAC Ni-IDA Kit plus columns	42165.01
SERVA IMAC Ni- and Co-IDA Test Kit	42166.01
SERVA IMAC Ni- and Co-IDA Kit plus columns	42167.01
SERVA IMAC Zn-IDA Test Kit	42168.01
SERVA IMAC Zn-IDA Kit plus columns	42169.01
SERVA IMAC Zn- and Cu-IDA Test Kit	42170.01
SERVA IMAC Zn- and Cu-IDA Kit plus columns	42171.01