BioTeZ IAC Kit

For manufacturing of Immuno Affinity Columns with BioTeZ-activated cellulose beads

Instruction

Kit for manufacturing of immuno affinity columns by immobilization of ligands with primary and secondary amines using activated cellulose beads

Order number: BTIK325005

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Note:

Read the instructions carefully before conducting the procedure!

Introduction

The BioTeZ-activated cellulose beads provide an easy way to immobilize ligands. The coupling reaction is simple to carry out. Many applications are possible, e.g. immobilization of proteins, peptides and nucleic acids. Precondition for coupling is that ligands contain primary and secondary amines.

BioTeZ-activated cellulose beads enables customized immuno affinity gels and columns with the following properties:

• reusable,
• excellent flow behaviour without extra pressure,
• little abrasion.
The coupled product has a high chemical stability to commonly used aqueous solutions. Usable with non-ionic detergents, denaturing solvents, e.g. 6 M guanidine hydrochloride and 8 M urea. Stable in organic solvents, such as 50% dioxane and 50% dimethylformamide.
To autoclave is not recommended.

Note: Alternatively for self conducting BioTeZ offers customized coupling service and column filling (Order number: BTKS324005).

Intended use

The intended use of BioTeZ IAC Kit is to manufacture individual immuno affinity gel or columns for immobilization of ligands containing primary and secondary amines to activated cellulose beads. The kit contains an instruction, activated gel, reagents and empty columns.
Immuno affinity chromatography is for example a helpful technology for purification or enrichment of analytes.

Characteristics of BioTeZ cellulose beads

Coupling capacity: ≥ 15 µmol/mL
Spacer: None
Particle size: 100-250 µm

Characteristics of immuno affinity columns with BioTeZ cellulose beads

max. flow rate: ca. 3 mL/min at room temperature
pH – long term stability: 3–10
pH – short term stability: 2–12, e.g. for reconstitution

Kit Components

Components	Volume	Code	Colour of top cap
BioTeZ activated cellulose beads	1 x 2 mL Gel (in Aceton)	BTIK325005a	white
Coupling solution (contains 0.05% Azid)	1 x 50 mL	BTIK325005b	black
Block solution (contains 0.05% Azid and 0.03% MIT*)	1 x 20 mL	BTIK325005c	red
Wash buffer 1 (contains 0,05% Azid)	1 x 20 mL	BTIK325005d	green
Wash buffer 2 (contains 0,05% Azid)	1 x 20 mL	BTIK325005e	yellow
storage buffer (contains 0,05% Azid and 0,03% MIT)	1 x 20 mL	BTIK325005f	blue
Empty columns, size: 3 mL incl. bottom frits, top frits, top caps and plain luer locks	5 piece	-	-

* MIT (Methylisothiazolon , 2-Methyl-4-isothiazolin-3-on)

The kit must be stored at a temperature of 2-8°C until it is used.
All kit components are designed for single use only

Other materials and equipment required

Distilled water
Glass frit (G1 or G2), filter flask, pump/water-jet pump
Rotating mixer (recommended)

Coupling procedure

A) preparing of the ligand solution

Before starting the coupling prepare the ligand solution.

Note! Use only the kit components (coupling solution) for preparing the ligand solution.

Note! If the ligand is kept in a buffer with many amino groups it is strongly recommended to change the buffer (rebuffering, dialysis, gel filtration) since these will couple to the gel and hence will reduce the coupling capacity of the gel.
For an optimal coupling efficiency the recommended relations between ligand and volume of activated gel is:
For a typical adsorbent: 0,1-2 µmoles ligand per ml activated gel
For protein ligands: 1-5 mg protein per ml activated gel
For high ligand concentrations the concentration can be higher.
Note!: If the ligand concentration is too high steric hindrance can occur.

For a standard coupling procedure the volume of coupling solution should be between 4 and 8 fold gel volume.

B) Gel preparation

BioTeZ-activated cellulose beads are supplied in acetone. Acetone must be drip off using a glass frit G1 or smaller.

The organic solvent must be washed away **completely** with water (5 times with at least the twofold gel volume within 5 minutes) before coupling the ligand.

Note! These wash procedure must be finished within 5 minutes.

Small gel volumes could be conducted directly in the columns (component of the kit). This includes wash and coupling.

C) Coupling

1. After wash add the activated gel to the prepared ligand solution. Use a portion of the coupling solution to spill the activated gel into the ligand solution.

   Note: The ligand solution must be added within 5 minutes after the first wash with water.

2. Rotate the mixture slowly overnight at room temperature. Recommended is a rotating mixer. Other gentle stirring methods may be employed. Magnetic stirrers are not recommended as these can damage the cellulose beads

3. Wash with at least 2 gel volumes of coupling buffer by using a frit or the kit components.

4. Remaining active groups: block the gel in slowly rotation for 2 hours with the two-fold gel volume block solution.

5. The gel is to wash soundly with at least three alternating cycles of wash buffer 1 and wash buffer 2. Recommended is to wash with at least 5 gel volumes of each buffer followed by a wash with storage buffer.

After this procedure the coupling is finished. The coupled gel could be stored at 2+8°C. Avoid that the gel is running dry.

Filling the column

After finalising coupling gel could be filled in columns together with storage buffer. Prepare the columns by using frits and luer lock. The top frit is optional. We recommend to use it carefully after sedimentation of the gel. Don't press on the top frit with pressure on the gel. To avoid the gel is running dry use the top cap and an exceeding storage buffer volume of ca. 1mL.

Annotations about immuno affinity chromatography

Immuno affinity usually has three steps: Binding, wash and elution. The conditions depend on your ligand and the analyte.
With proteins like antibodies typically binding step and wash is performed in PBS or similar buffer. Elution is usually done using methanol (mostly with haptens) or citrate buffer pH 2.6 (proteins).
Depending on the affinity the flow rate for binding and elution should be chosen leisurely. Usually it is not higher than 1 mL/min.

Important points and precautions

1. The kit must be conducted solely in accordance with these instructions, which contain the necessary instructions for use and warnings. Any modification to the kit are not authorised by the manufacturer, including with regard to its procedure or the reagents and materials used, is prohibited.
2. The manufacturer assumes no liability and indicates that the user is solely responsible for the consequences of any alterations made, for non-observance of instructions or for performing the kits without paying due attention.
3. The equipment used must be maintained in accordance with the manufacturers' instructions and any applicable guidelines. Before equipment is used it should be checked for fault-free operation.
4. The materials and reagents included in the kit are intended for single use only. Excess material and materials and reagents that have exceeded their expiry date/lifetime should be disposed of correctly. You should observe the regulations that apply to you.
5. Do not perform the kit if the packaging or contents are damaged.
6. The kit may only be carried out by trained specialists. Pregnant women should not perform the kit.
7. The kit is intended for use at room temperature (20 to 25°C). Deviations in the climatic conditions can negatively influence the results.
8. You should exclusively use the materials and reagents included in the kit. Do not mix these with materials and reagents from other kits, even where such kits are from the same manufacturer and for the same purpose. Similarly, the use of materials and reagents from other manufacturers instead of those contained in the kit is forbidden.
9. Ensure that the materials, equipment and reagents are clean.
10. Before use, check the materials and reagents for any visible contamination.
11. Observe general health and safety regulations.
12. Follow the instructions for this kit very closely. The washing procedure, in particular, incomplete removing of Acetone is a source of error if the washing is not performed adequately and in time line.
13. Note that waste should be collected and disposed properly. Pay attention to the regulations that apply to you. Disinfect thoroughly.
14. The kit contains substances such as Azit and MIT for conservation that are toxic. Avoid contact with eyes and skin! Wear protective gloves!
15. When disposing of the materials and reagents, observe any potential harm they may cause to the environment. Observe the regulations that apply to you.
16. Observe the fundamentals of good laboratory practice.
17. Observe safety regulations, e.g. do not eat, drink or smoke in the workplace; keep materials and reagents away from foods and feeding stuffs; wear protective clothing (lab coat, safety glasses and gloves).
18. In the event of a warranty claim the entire kit should be returned to the manufacturer, BioTeZ Berlin-Buch GmbH, within 14 days with a written explanation.

For further information, please contact
BioTeZ Berlin-Buch GmbH
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www.biotez.de