KNAUER

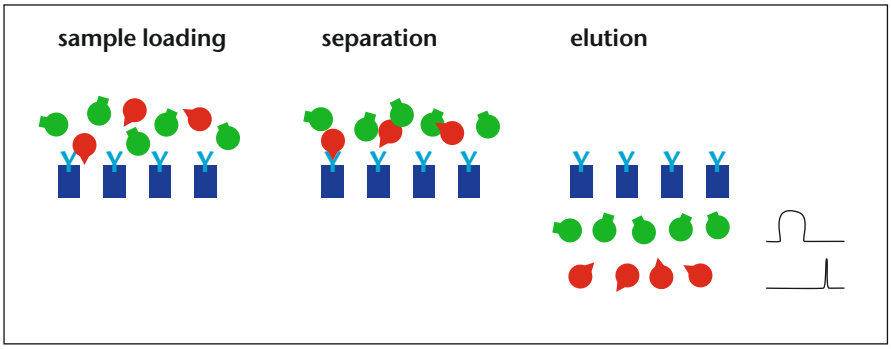
Affinity chromatography (AC)

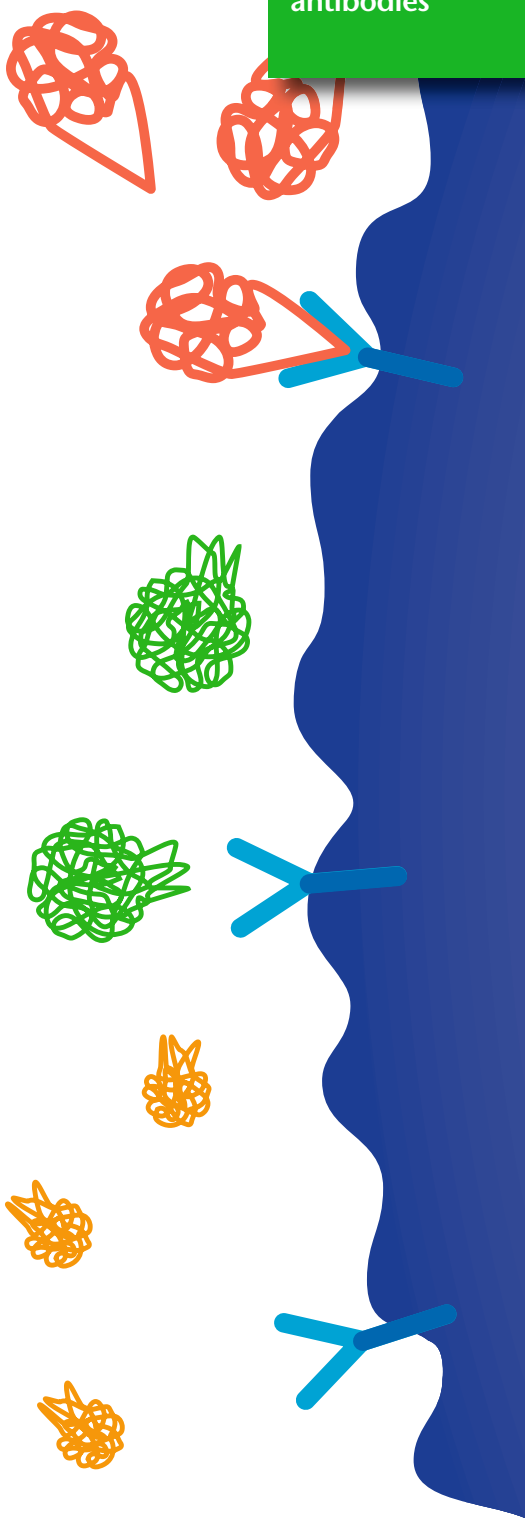
purification of specific proteins like antibodies

BioFox 40 ACT

Analytical and preparative separations of proteins

Pre-activated media for preparative and bioprocess scale affinity chromatography with user's choice of ligands





Affinity chromatography separates proteins on the basis of a reversible interaction between a protein and a specific ligand covalently bound to a pre-activated chromatographic medium. Thus, the typical affinity chromatography is often referred to as "lock and key principle" of a part of a molecule (key) with an immobilized ligand (lock). Antibodies, antigens, enzymes, short nucleic acids or peptides can be used as affinity ligands. They are coupled via their reactive functional groups such as amino, carboxyl, hydroxyl of thiol moieties to the gel. In particular, antigens or antibodies as ligands create a highly selective media for immunoaffinity purification. After loading the protein mixture into the separation column, non-binding molecules are almost entirely eliminated using rinse buffers, so that the final elution buffer contains the target molecule in high purity. If a specific ligand is available, classical affinity chromatography is considered as a 2-step separation process for high purity target molecules because a second step is necessary to remove unwanted small molecules, such as salts or aggregates.

Pressure stability up to 40 bar (580 psi) – fast and high resolution biochromatography

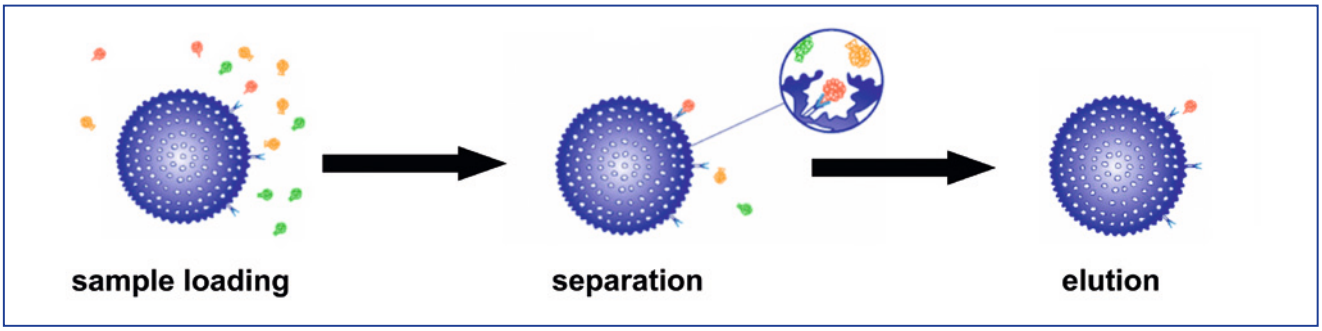
BioFox 40 ACT pre-activated separation media is produced from agarose using a proprietary cross-linking method that results in a highly porpous and physically stable matrix. Besides the well-known selectivity of agarose, BioFox 40 ACT is pressure resistant up to 40 bar (580 psi) for high throughput biochromatography.

BioFox 40 ACT is activated according to the bromhydrin method. This activation method is, although proprietary, based upon well-understood chemistry, allowing you to perform the coupling reaction in an aqueous solution.

Step 1: Preparation of an immunoadsorbent with BioFox 40 ACT

Matrix-$OCH_2CH(OH)CH_2Br$ + Nu^- (e.g. –SH, $–NH_2$ or –OH) ——> Matrix-$OCH_2CH(OH)CH_2$-Nu
(Nu = Nucleophiles)

Step 2: Coupling reaction in aqueous solution



Since no toxic chemicals are necessary for the coupling procedure and the BioFox ACT products are stable at room temperature, coupling can easily be performed on your bench and at room temperature.

BioFox 40 ACT

- Made from agarose, well-established and well-known in the biotechnology industry
- Simple coupling procedures at room temperature
- Stable at room temperature in aqueous solution and at neutral pH
- Suitable for coupling of ligands containing sulfhydryl, amino or hydroxyl groups
- Optimum particle size of 40 µm and narrow particle size distribution for high throughput preparative and bioprocess scale applications

Coupling conditions and selection of coupling buffers

BioFox 40 ACT comes ready to use. Proteins or other biomolecules with free amino and thiol groups will easily couple to BioFox 40 ACT. Just add the ligand to the suspension, stir and incubate overnight.

Hydroxyl groups can also be used for coupling, but will require pH 12 which is not compatible with most proteins. However, stable molecules can be coupled using the hydroxyl group. Remaining reactive groups are deactivated using 2-mercaptoethanol or ethanolamine.

Stability

BioFox 40 ACT media is stable for 12 months in aqueous solutions containing 22% ethanol at neutral pH and at room temperature without any significant decrease of coupling activity. The choice of storage buffer for a coupled gel medium depends on the properties of the ligand.

Type of ligand	Functional group of ligand	Coupling buffers
Organic molecules, peptides	Thiol-SH	pH 7 and higher. Sensitive ligands can be coupled at pH 7 but a better yield is achievable at higher pH. Basicity of the ligand will determine the coupling pH.
Organic molecules, peptides	Amines: primary ($-NH_2$) secondary (-NHR) tertiary ($-NR_3$)	When the ligand is used in excess, dissolve the ligand in distilled water and the basicity of the ligand will determine the coupling pH.
Proteins, polypeptides	Thiol (-SH)	pH 7 and higher. Sensitive ligands can be coupled at pH 7 but a better yield is achievable at higher pH.
Proteins, polypeptides	Primary amines ($-NH_2$)	Coupling yield will increase at higher pH. A carbonate buffer of pH 8 to 8.5 often gives sufficient coupling without denaturation of sensitive polypeptides and proteins. Another possibility is to run the coupling reaction at lower temperature.
All types	Hydroxyl (-OH)	The low nucleophilicity of the hydroxyl group requires coupling conditions at very high pH > 12. At a pH < 12 cross-linking and hydrolysis will compete with the coupling procedure.

The Bioline Rack – with glass columns

Media characteristics	BioFox 40 ACT
Exclusion limit [kDa]	1 200
Max flow rate at 20 cm bed height [cm/h]	> 500
Particle size [µm]	32 – 60
Spacer arms (# Atoms)	4 – 16
Agarose content [%]	7.4 – 7.8
Coupling groups	-OH, -NH_2, -SH
Degree of substitution [mol/mol]	0.6 – 0.7
Solvent stability	100 % methanol, 100 % ethanol, 8 M urea, 6 M guanidine hydrochloride, 30 % acetonitrile, 70 % formic acid, 30 % trifluoroacetic acid

Column specifications	BioFox 40/10000
Exclusion limit [kDa]	10 000
Max flow rate at 8x300 mm bed height [cm/h]	> 10
Particle size [µm]	32 – 60
Spacer arms (# Atoms)	4 – 16
Agarose content [%]	4.6 – 5.0
Coupling groups	-OH, -NH_2, -SH
Degree of substitution [mol/mol]	0.6 – 0.7
Solvent stability	100 % methanol, 100 % ethanol, 8 M urea, 6 M guanidine hydrochloride, 30 % acetonitrile, 70 % formic acid, 30 % trifluoroacetic acid

Ordering information	
Article No.	**Column size/volume**
Y4016	Bulk media, 50 ml
Y4006	Bulk media, 300 ml
Y4086	Bulk media, 1 l
Y4096	Bulk media, 5 l
Y4076	Bulk media, 1 l (40/10000)

BioFox 40 ACT and BioFox 40/10000 media are supplied as an aqueous suspension with 22 % ethanol as preservative and is immediately ready for use after washing.

Technical data are subject to change without notice. Please check our website for latest updates and changes.

Visit www.knauer.net for more information on KNAUER's complete range of biochromatography products.

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