

# WorkBeads™40 ACT



## Activated Media for Preparative and Bioprocess Scale Affinity Chromatography with User's Choice of Ligands

- 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 Sulphydryl, Amino or Hydroxyl Groups

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### Media Description

**WorkBeads™40 ACT** pre-activated separation media are produced from agarose using a proprietary cross linking method that result in a highly porous and physically stable agarose matrix. Agarose based matrices have been successfully used for decades in biotechnology research and in the industrial purification of proteins. Agarose is proven to be exceptionally compatible with natural biomolecules such as proteins, DNA, carbohydrates etc. The material shows minimal non specific interaction due to the hydrophilic nature of agarose. Unlike matrices made from synthetic polymers, agarose does not have micro pores that can contribute to local pH variations in the micro-environment in the column which lead to distorted separations.

**WorkBeads 40 ACT** is activated according to the Bromohydrin method. This activation method is proprietary and based upon well known chemistry and allows you to perform the coupling chemistry in aqueous solutions.

Matrix-OCH<sub>2</sub>CH(OH)CH<sub>2</sub>Br + Nucleophil (i.e. -SH, -NH<sub>2</sub> or -OH) --> Matrix-OCH<sub>2</sub>CH(OH)CH<sub>2</sub>-Nu

**WorkBeads 40 ACT** is supplied as an aqueous suspension with 22% ethanol as preservative. After washing, the gel is immediately ready for use. As no toxic chemicals are involved and the **WorkBeads™40 ACT** products are stable at room temperature the coupling procedure can, as long as your application so allows, easily be performed on your bench and at room temperature.

### Applications

**WorkBeads 40 ACT** is ready for use.

Proteins or other molecules with free amino and sulfhydryl groups will couple easily to **WorkBeads 40 ACT**. Just add the ligand to the suspension, stir and incubate over night.

Hydroxy groups can also be used for coupling but will require pH above 12 which is not compatible with most proteins. However, stable molecules could be coupled using the hydroxyl group. Remaining reactive groups are deactivated using mercapto-ethanol or ethanol-amine

## Separation Media Characteristics

<b>Agarose Content %</b>	7.4 - 7.8
<b>Exclusion Limit</b>	1200 kD
<b>Max Flow Rate at 20cm Bed Height and 5 Bar, cm/h</b>	> 500
<b>Particle Size, <math>\mu\text{m}</math></b>	32 - 60
<b>Spacer Arms (# Atoms)</b>	4 - 16
<b>Degree of Substitution (mol/mol)</b>	0.6 - 0.7
<b>Coupling Groups</b>	-OH, -NH <sub>2</sub> , -SH
<b>Solvent Stability After Coupling the Ligand</b>	100% methanol, 100% ethanol, 8 M urea, 6 M guanidine hydrochloride, 30% acetonitrile, 70% formic acid, 30% trifluoroacetic acid

## Coupling Conditions and Selection of Coupling Buffers

<b>Type of Ligand</b>	<b>Functional Group of Ligand</b>	<b>Coupling Buffers</b>
Organic molecules, peptides	Sulphydryl (-SH)	pH 7 and higher. Sensitive ligands can be coupled at pH 7 but a better yield will be obtained at a higher pH.
Organic molecules, peptides	Amino (-NH <sub>2</sub> ) R <sub>2</sub> -NH R <sub>3</sub> -N	When the ligand is used in excess, dissolve the ligand in distilled water and let the basicity of the ligand determine the coupling pH
Proteins polypeptides	Sulphydryl (-SH)	pH 7 and higher. Sensitive ligands can be coupled at pH 7 but a better yield will be obtained at a higher pH
Proteins polypeptides	Primary amino (-NH <sub>2</sub> )	Coupling yield will increase at higher pH. A carbonate buffer of pH 8-8.5 gives often 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 demands coupling condition at very high pH (pH > 12). At a pH > 12 cross-linking and hydrolysis will compete with the coupling procedure

## STABILITY

**WorkBeads 40 ACT** media is stable at neutral pH and at room temperature in aqueous solutions containing 22% ethanol (as preservative) for one year, without any significant decrease in the coupling activity. The choice of storage buffer for a coupled gel depends on the properties of the ligand.

## ORDERING

Product Name	Volume	Article Number
<b>WorkBeads™ 40 ACT</b>	Bulk Media – 50 ml	<b>40 400 001</b>
<b>WorkBeads™ 40 ACT</b>	Bulk Media – 300 ml	<b>40 400 003</b>
<b>WorkBeads™ 40 ACT</b>	Bulk Media – 1 L	<b>40 400 010</b>
<b>WorkBeads™ 40 ACT</b>	Bulk Media – 5 L	<b>40 400 050</b>

All media are preserved in 22% ethanol.

**To purchase Bio-Works separation media contact your local distributor. Alternatively you may email, fax or phone Bio-Works directly at:**

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