Instructions for use

WorkBeads 40 ACT

Pre-activated separation media for coupling of your choice of ligand

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

UNPACKING AND INSPECTION

Unpack the shipment as soon as it arrives and inspect it for damage. Promptly report any damage or discrepancies to your local supplier or directly to Bio-Works Co. Ltd.

STORAGE

WorkBeads 40 ACT gels are supplied as aqueous suspensions containing 22% ethanol as preservative. The gels are stable at pH 7 and at room temperature for one year, without any notable decrease in coupling activity. The choice of buffer for storage of a coupled gel depends on the properties of the ligand.

HOW TO USE

The coupling procedure is very simple. Exchange the ethanol solution with your selected coupling buffer, add the ligand and leave the coupling reaction to take place at room temperature overnight.

1 Preparing the gel.
- Pour the required volume of gel slurry on a glass filter.
- Wash three times with three gel volumes of distilled water under suction. Stir carefully with a soft spatula.
- After the third washing allow the gel cake to crack.
- Weigh the gel and transfer it to the reaction bottle.

Note! The amount of “dried” gel in grams, treated as above, corresponds roughly to the same amount of packed gel in ml.

2 Selecting the buffer.
When selecting the coupling buffer the nucleophilicity of the ligand to be coupled has to be considered. Different coupling buffers are recommended for the functional groups sulphydryl, amino and hydroxyl. See Table 1.

Note! The coupling buffers should not contain any nucleophilic components e.g. Tris, glycine, since these will couple to the gel.

3 Coupling reaction.
- Dissolve the ligand to be coupled in the selected coupling buffer. Dilute with coupling buffer to a volume of twice the volume (~g) of “dried” gel.
- Pour the gel in the coupling buffer containing the ligand.
- Agitate gently, avoiding magnetic stirring (utilize the end to end method), overnight at room temperature or if required at a lower or a higher temperature.

4 Washing procedure of coupled gel
- Transfer the ligand-coupled gel to a glass filter.
- Wash with distilled water until all excess of ligand is removed.
- If you want to block suspected remaining active groups proceed to 5, if not wash with three volumes of the buffer selected for the chromatographic step.

5 Blocking of remaining active groups
- Add an excess of ethanolamine or mercaptoethanol to the coupled gel, for blocking of remaining active groups on the gel.
  Example: 1 M ethanolamine solution is adjusted to the required pH with 1 M hydrochloric acid (HCl). Add the solution to twice the amount of coupled gel.
- Agitate gently over night, avoiding magnetic stirring (utilize the end to end method), at room temperature or at the conditions required by your ligand.

6 Washing procedure of coupled and blocked gel
- Transfer the blocked gel to a glass filter.
- Wash with distilled water until all excess of blocking agent is removed.
- Wash with three volumes of the buffer selected for the chromatographic step.

The coupled and blocked gel is now ready for use.

Table 1
SELECTION OF COUPLING BUFFERS

Type of ligand	Functional group of ligand	Coupling buffers
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 ($-NH_2$) R_2-NH R_3-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 ($-NH_2$)	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