Instructions for use

Roti®-MagBeads COOH

HP58

Activated magnetic silica gel beads for covalent coupling of chosen ligands. Ready-to-use.

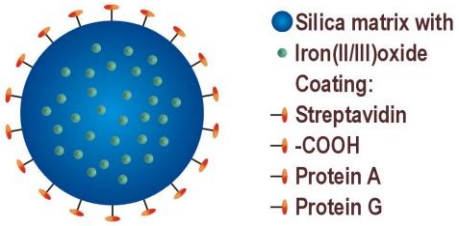

Fig. 1: Composition of Roti®-MagBeads

- Easy and standardized handling
- Fast and easy batch format without columns
- Allows harsh pH values and high ionic strength
- For ligand coupling prior to purification of biomolecules, immunoassays , and cell sorting
- Well suited for automated applications.

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I. Introduction

Roti®-MagBeads COOH is a matrix for simple and efficient coupling of any chosen ligand that can be coupled to carboxylic groups, such as antibodies, proteins and oligonucleotides. Subsequently, the coated beads can be used for isolation of proteins and peptides, nucleic acids and other biomolecules, and for positive or negative cell sorting. In case antibodies are coupled to the beads, immunoassays can be carried out.
The magnetic properties enable easy and quick washing steps, allowing one preparation to take place in approx. 10 mins. The small bead size of 1 µm in average provides a large surface with excellent coupling capacities. Handling is easy and identical to standardized protocols for beads of other suppliers, providing easy establishment of the assays. The high ligand coupling capacity combined with low non-specific binding minimizes the signal-to-noise ratio. Subsequent assays are well reproducible with high recovery rates.

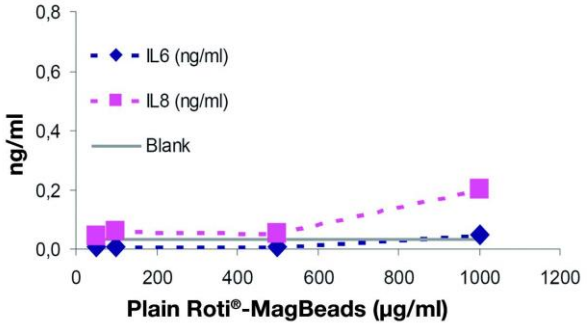

Fig. 2: Biocompatibility of Roti®-MagBeads. Incubation of lung cells (A549) (shown here) and human whole blood with plain Roti®-MagBeads. Cell stress was measured through release of IL6 and IL8. No increase of IL6 and IL8 was measured with magnetic beads of up to 500 µg / ml.

The Matrix of Roti®-MagBeads COOH consists of uniform, superparamagnetic silica beads coated with activated surface (-COOH). In those beads, nanometer-sized particles of magnetic iron(II/III)oxide are embedded in silicium oxide, resulting in high magnetic mobility with low aggregation potential.
The surface is made from pure activated silicium oxide and allows application of harsh pH values as well as of media with high ionic strength.

II. Parameters

Concentration	10 ± 0,5 mg / ml
Size distribution	0,7 – 1,4 µm
Mean particle sizing (CI90%)	1,0 ± 0,05 µm
Bead number	6-12 x 10^9 Beads / ml
Iron content	30-40 %

Suspension in PBS (pH 7.4), 0.05 % sodium azide.

III. Application

III.a Pretreatment

Vortex bead suspension well before use.
Rinse the beads once with water as follows to remove the NaN_3.

1. Resuspend beads by shaking/vortexing
2. Pipette needed amount in tube or micro plate
3. Collect beads by placing the tube or micro plate on the magnet for 1-2 mins.
4. While tube/micro plate is still on the magnet, carefully remove supernatant without touching the pellet of beads
5. Take tube/micro plate from the magnet and add 1 to 4 initial volumes of washing buffer or water.
6. Resuspend beads by vortexing or pipetting in MES (see III.c).

III.b Covalent Coupling Protocols on Roti®-MagBeads COOH by Carbodiimide Method

This protocol describes the covalent coupling of amino-group containing ligands such as antibodies, proteins or low molecular substances to Roti®-MagBeads COOH activated beads using carbodiimide method. Carbodiimides react with the terminal carboxylate-groups from the magnetic beads to highly reactive O-acylisourea derivatives and react readily with amino-groups of the ligands.
Protein binding is performed via amine groups (from lysine and/or as unblocked N-termini). In general, the amount of protein which is immobilized to the beads should be optimized by the customer according to the proposed targets. The higher the amount of antibody / protein per milligram of beads, the higher the degree of surface coating with the protein and implicitly the higher the activity is obtained. For antibodies / proteins, we recommend to use a minimum amount of 50 µg antibody/protein per 1 mg Roti®-MagBeads COOH.

Note:

- All buffers used for activation or coupling may not contain amino-groups, proteins or high ionic strength conditions.
- We recommend preparing the EDC solution immediately before use, adding it rapidly to the reaction tube.
- For an optimal binding capacity of the molecules of interest it is possible to optimize the pH value between pH 4.0 - 6.5 of the Washing & Binding Buffer.
- The protocol describes the coupling of biomolecules on 1 ml (10 mg) beads particles. It can be scaled up by adjusting volumes of required reagents.

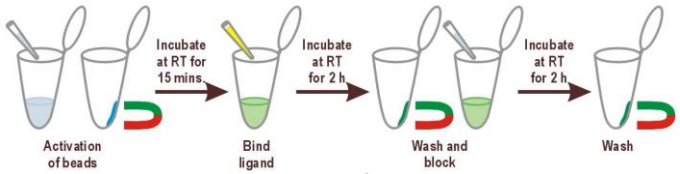

Fig. 3: Application scheme of Roti®-MagBeads Streptavidin

Activation

1. Wash the Roti®-MagBeads COOH 2 x with 1 ml MES buffer as described above (see III.b) and discard the supernatant by using the magnetic separation.
2. After the second wash step resuspend the magnetic beads in 500 µl MES buffer containing 20 mg EDC and 20 mg NHS and mix on a shaker for 15 minutes at room temperature.
   Note: EDC/NHS solution in MES has to be freshly prepared!
3. Discard the supernatant using the magnetic separation and wash the beads 1x with MES. Discard the MES buffer. The beads contain now activated COOH groups that can bind proteins/amine containing molecules.

Ligand binding

4. Add amine group containing ligands in a total of 500 µl Coupling Buffer with a protein concentration of 1 to 5 mg/ml (e.g. 50 µg protein/mg beads) to the activated beads. Mix the beads suspension on a shaker for 2 hours at room temperature.
5. Wash the particles 3 x with 1 ml Washing Buffer for coupling.
6. Resuspend the particles in blocking buffer. Mix the bead suspension on a shaker for 2 hours at room temperature.
7. Wash the particles 3 x with 1 ml Washing Buffer for coupling and 3 x with 1 ml pure PBS. Discard the supernatant.
8. Re-suspend the beads in Storage Buffer. They are ready to be tested for specific activity and to be applied to ligand-coupling assays.

IV. Sanetisation of Roti®-MagBeads

For sanetisation of Roti®-MagBeads we recommend to wash beads repeatedly (3-5 times) in ethanol (96 % or 100 %) according to the protocol given under III.b. Wash the beads then once in sterile buffer and use for cell assays.
Beads treated by that procedure can be used for all cell assays with cell samples. Further cultivation of the cells, however, may be hindered by residual bacteria or endotoxins

V. Storage:

Store at +4 °C. Do not freeze!
Store beads in well closed vial and in upright position to prevent drying of the beads. Drying makes them more difficult to resuspend and may decrease their activity.
Vortex bead suspension well before use.

VI. Abbreviations:

EDC: N-(3-dimethyl aminopropyl)-N'-ethylcarbodiimide-hydrochloride
NSH: N-hydroxy succimide

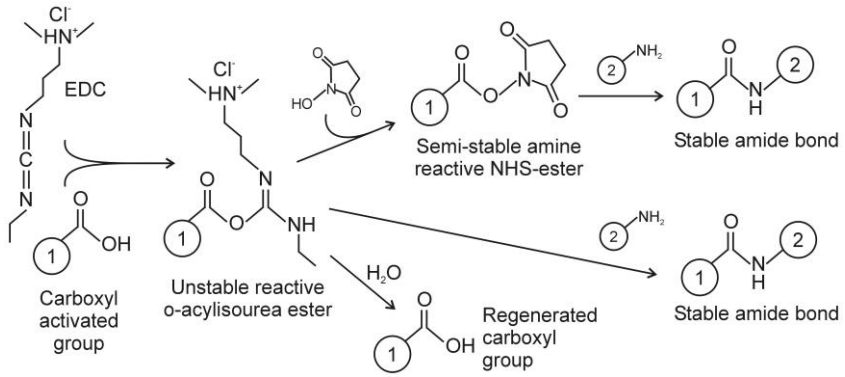

Fig. 4: Reaction mechanism of activation and ligand binding

VII. Additional material required

- Magnets for bead separation/collecting.
- Washing Buffer for Activation: 0.1 M 2-(N-Morpholino)ethane sulfonic acid (MES), pH 5.0
- Activation Buffer: MES with 40 mg / ml 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and 40 mg / ml N-hydroxysuccinimide (NHS)
  Note: Always prepare Activation buffer freshly !!!
- Coupling Buffer: PBS, pH 7.4 (Roti-Stock 10 x PBS, Art. No.: 1058.1)
- Washing Buffer for coupling: PBS, pH 7.4, 0.05 % Tween 20 (Roti-Stock 10 x PBST, Art. No.: 1059.1)
- Blocking buffer: 0.1 M Tris buffer
- Storage Buffer PBS, pH 7.4, 0.05 Tween 20, (optional: 0.1 % BSA), 0.05 % sodium azide (Roti-Stock 10 x PBST, Art. No.: 1059.1, add BSA and Na-azide)
- Round bottom 96-well plates
  Rotilabo®-Micro assay plates, Art. No.: 9291.1
  Deep well plates, Art. No.: EN06.1
  or reaction tubes
  Rotilabo® reaction tubes 500 µl, Art. No.: EA83.1
  Rotilabo® reaction tubes 1.5 ml, Art. No.: EA84.1
  or thin walled reaction tubes
  Mµlti®-Ultra tubes 0.2 ml, Art. No.: H560.1
  Mµlti®-Ultra tubes 0.65 ml, Art. No.: A774.1

Roti®-MagBeads COOH	
HP58.2	**50 µl**
HP58.1	**2 ml**