

Carboxyl-Adembeads Coupling Kit 02820

For research use only

INTRODUCTION

The kit is based on the use of the **Biomagnetic separation technology**. The separation method is gentle and does not require the use of columns or centrifugation step.

Biomagnetic separation technology is a simple technique based on the separation of **superparamagnetic beads** using a magnetic field. When added to a complex medium, the magnetic beads will bind to the target. This interaction is based on the specific affinity of the ligand on the surface of the beads. The resulting target-bead complex can be removed from the suspension using a magnet. The benefits of magnetic handling are easy washing, separation and concentration of the target without any need for centrifugation or columns.

Superparamagnetic beads exhibit magnetic properties only when placed within a magnetic field and show no residual magnetism when removed from this field.

COUPLING KIT PRINCIPLE

Carboxyl-Adembeads are monodispersed and superparamagnetic nanoparticles composed of magnetic core encapsulated by a highly cross-linked hydrophilic polymer shell. The surface is activated with carboxylic acid functionality. The hydrophilic surface ensures low non-specific binding, excellent dispersion abilities and easy handling of the beads in a wide range of buffers. Carboxyl-Adembeads are designed to act as solid support for a wide variety of biomagnetic separations and manipulations.

Proteins, oligonucleotides or other target specific molecules can be easily covalently coupled directly onto the surface of Adembeads via primary amino groups. Once coupled with ligand, the beads can be added to a cell lysate or other suspensions containing your target molecule. After a short incubation, the beads can be pulled

Version 1.1

to the side of the test tube by use of a magnetic device allowing aspiration of unbound material. Furthermore, the magnet facilitates washing and concentration of the isolated target.

PRODUCT DESCRIPTION

Carboxyl-Adembeads are produced under aseptic conditions and are sold in an aqueous suspension containing 0.09% NaN₃.

The reagents are provided for at least 10 coupling each of 1 mg of Carboxyl-Adembeads of each size.

Amount	Component	Storage
1ml	Carboxyl-Adembeads, 200nm, 1%	+ 4°C
1ml	Carboxyl-Adembeads, 300nm, 1%	+ 4°C
1ml	Carboxyl-Adembeads, 500nm, 1%	+ 4°C
60mg	EDC	- 20°C
15 ml	Activation Buffer (10X)	+ 4°C
15ml	Storage Buffer (10X)	+ 4°C

Table 1: Reagents provided with the kit

The concentration of the supplied beads is 10mg/ml.

Calculation:
1mg beads = 100µl.



EDC must be stored at -20°C

PROTOCOL SUMMARY

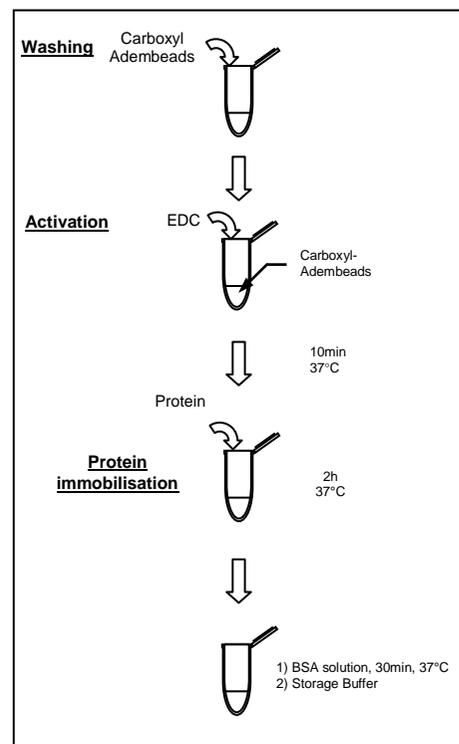


Figure 1: Protocol overview

INSTRUCTION FOR USE

The functional carboxylic acid groups of Carboxyl-Adembeads offer the possibility for many different immobilisation procedures for use with proteins or other ligands via EDC activation for example.

We recommend coupling 10-50 µg of ligand per mg beads. **The suggested protocol described below illustrates an example using 1 mg of beads**, but should be scaled up or down to suit specific needs. Protocols should be optimised to meet your requirements (e.g. sample volume, concentration of ligand / beads/ EDC).

A) Before starting

Dilute Activation Buffer (10X) and Storage Buffer (10X) 10 fold in distilled water.

B) Washing procedure for Carboxyl-Adembeads

1. Transfer 100µl of magnetic beads into the desired test tube and place the tube in a magnet for 1min.
2. Pipette off the supernatant carefully, leaving beads undisturbed.
3. Remove the test tube from the magnet and resuspend the beads carefully with 100µl of Activation Buffer (10X diluted in distilled water).
4. Repeat steps 2-3.

C) Coating procedure using EDC activation

The Carboxyl-Adembeads can be activated with EDC (1-ethyl-3-(3-dimethylamino propyl) carbodiimide hydrochloride, MW 191.7) that reacts with the carboxylic acid groups to form an amine-reactive intermediate.

Activation Step:

1. Prepare a 4mg/ml EDC solution in Activation Buffer (1X, diluted in distilled water) and add 80µl of EDC solution per 100µl of beads. Vortex to mix properly.
2. Incubate for 10 min at 37°C under shaking.

The beads are now activated and ready for coating with a ligand.

Note: Depending on the ligand, protocol can be customised (incubation time / concentration of EDC).

Protein immobilisation

1. Add 10-50µg of proteins per mg of activated particles.

Note: For best results, we recommend working with a protein solution at 1-2 mg/ml. Dilute protein solution in Activation Buffer (1X) is possible. The protein solution must be free of primary amines (e.g. Tris buffer) and others proteins.

2. Incubate for 2h at 37°C under shaking.
3. Prepare the Bovine Serum Albumine (BSA) solution in Activation Buffer (0.5mg/ml) and add 200µl of BSA solution to 100µl of Protein-coated beads. Vortex to mix properly.

Note: Depending on the ligand, protocol can be customised (Ethanolamine / Tris..).

4. Incubate for 30min at 37°C under shaking.
5. Wash the beads with the Storage Buffer twice and resuspend the beads at the desired concentration.

ADDITIONAL MATERIAL REQUIRED

- Magnetic device
- Rotation device
- Test tubes
- Related products: Magnetic Devices
 - Adem-Mag SV, 1.5 ml (# 20101)
 - Adem-Mag MV, 15 ml (# 20102)
 - Adem-Mag HV, 50 ml (# 20103)

STORAGE/STABILITY

When stored in unopened vials at 2-8°C, Carboxyl-Adembeads are stable until expiration date printed on the label.

The Carboxyl-Adembeads must be maintained in liquid during storage and all handling steps. Drying will result in reduced performance. Do not freeze the product.

PRECAUTIONS

Precautions should be taken to prevent bacterial contamination of protein-coated Adembeads. If cytotoxic preservatives are added these must be carefully removed before use by washing.

WARNINGS AND LIMITATIONS

For in vitro research only. Not for use in human diagnostic or therapeutic procedures.

Sodium azide is toxic if ingested. **Avoid pipetting by mouth.** Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. When disposing through plumbing drains, flush with large volumes of water to prevent azide buildup.

WARRANTY

The products are warranted to the original purchaser only to conform to the quality and contents stated on the vial and outer labels for duration of the stated shelf life.

Ademtech's obligation and the purchaser's exclusive remedy under this warranty is limited either to replacement, at Ademtech's expense, of any products which shall be defective in manufacture, and which shall be returned to Ademtech, transportation prepaid, or at Ademtech's option, refund of the purchase price. Claims for merchandise damaged in transit must be submitted to the carrier.

Ordering Information

Product Description	Code
Carboxyl-Adembeads Coupling Kit	02820

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