

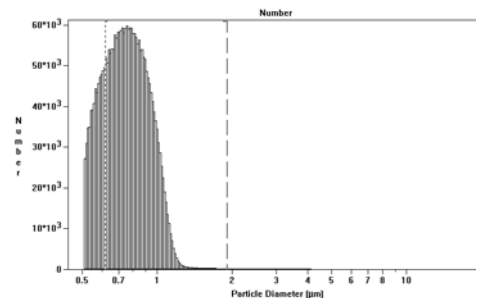


M-PVA C1x *for research only*

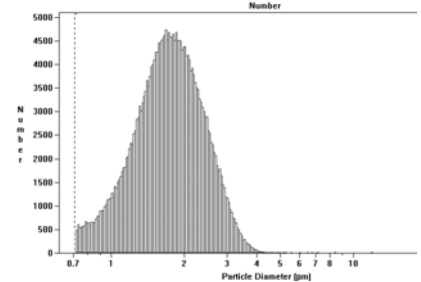
medium carboxylated M-PVA Magnetic Beads

Standard Bead Sizes¹:
*indicated by the last number (1 or 2)
in the product name*

M-PVA C11: 0.5 – 1.0 µm



M-PVA C12: 1.0 – 3.0 µm



Standard Package Size²: 10 ml bead suspension

Concentration: 50 mg/ml

Standard Magnetite Content: 50 - 60 %

Storage: at room temperature in H₂O

Carboxylation degree: **M-PVA C11:** 750 µmol COOH/g

M-PVA C12: 500 µmol COOH/g

Properties: The superparamagnetic beads consist of a matrix of polyvinyl alcohol, which is subsequently carboxylated for easy coupling of proteins, nucleic acids or other amino ligands with known coupling methods. The high content of magnetite permits a rapid separation-process. The beads have a polydispers size distribution.

¹ other beads sizes on request

² other package sizes or bulk ware on request



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Denmark for Chemagen**

www.md-scientific.dk - 7027 8565 - info@md-scientific.dk

Further Questions?

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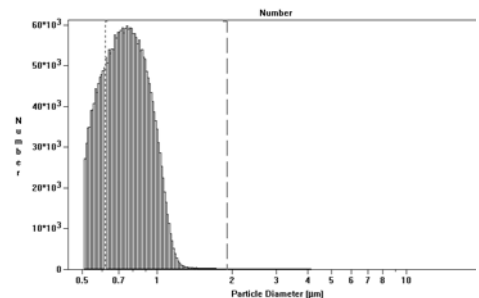


M-PVA C2x *for research only*

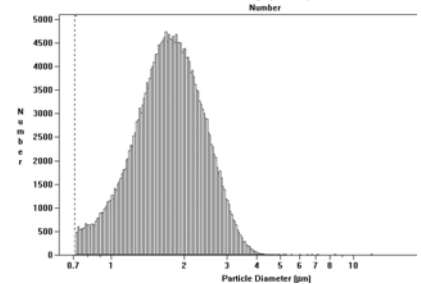
highly carboxylated M-PVA Magnetic Beads

Standard Bead Sizes¹:
*indicated by the number (1 or 2)
after the product name*

M-PVA C21: 0.5 – 1.0 μm



M-PVA C22: 1.0 – 3.0 μm



Standard Package Size²: 10 ml bead suspension

Concentration: 50 mg/ml

Standard Magnetite Content: 50 - 60 %

Storage: at room temperature in H₂O

Carboxylation degree: **M-PVA C21:** 1100 $\mu\text{mol COOH/g}$

M-PVA C22: 950 $\mu\text{mol COOH/g}$

Properties: The superparamagnetic beads consist of a matrix of polyvinyl alcohol, which is subsequently carboxylated for easy coupling of proteins, nucleic acids or other amino ligands with known coupling methods. The high content of magnetite permits a rapid separation-process. The beads have a polydispers size distribution.

¹ other beads sizes on request

² other package sizes or bulk ware on request

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Standard Coupling Protocol

The beads with COOH groups can be used to couple polymer or nonpolymer molecules, which contain amino groups. At first the COOH matrix must be activated. If the reaction is carried out in aqueous media, which is necessary for many biomolecules, we recommend the use of water soluble carbodiimides, e.g. the commercially available 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC). The reaction of COOH groups and EDC yields very reactive O-acylisourea intermediates, which react readily with the amino groups of other molecules [1].

For activation the pH-values should be kept between 4.7 and 6. Reactions above pH = 7.5 should be avoided (e.g. 0.1 M MES buffer for acidic pH-values and 0.1 M phosphate buffer for neutral pH-values).

The following protocol can be used to immobilise approx. 250 - 1000 μg^3 protein.

However, the binding capacities depend on the molecule of interest. Thus it is possible to optimize the choice of the buffer, the pH value and the ratio of reactants of this protocol.

1. Shake up the bead suspension well, take out 1 mL (50 mg/mL) and wash 2x with 0.1 M MES buffer pH 5.0 (activation buffer).
2. Add 2 mL of a freshly [2] prepared EDC/activation buffer solution (25 mg/mL), suspend the beads in this solution thoroughly and mix this suspension gently for 15 min.
3. Wash 1x with activation buffer and 1x with 0.1 M sodium phosphate buffer pH 7.5 (coupling buffer). The activated beads should be suspended in the washing buffers only for a short period of time, to avoid hydrolytic cleavage of the activated matrix.
4. Add 2 mL of a freshly prepared protein/coupling buffer solution (1 mg/mL) and mix thoroughly for at least 2 h at RT.
5. Mix thoroughly at least 2 h at RT and at least 12 h at 4°C.
6. Wash 2x with 4 ml coupling buffer.
7. Incubate at least 2 h in 0.05 M Tris buffer, that contains 0.1% ethanol amine (deactivation buffer; pH 7.5 - 8.5).
8. Wash 3x with storage buffer, suspend in this buffer the concentration desired and store at 4°C.

³ the binding capacity per mass unit generally decreases with increasing average bead size; the use of M-PVA C2x beads, which have a very dense COOH loading, usually leads to higher binding capacities for smaller molecules.

Further Questions?



The buffers used for activation or coupling must not contain any amino groups. An EDC concentration of 0.1 - 0.5 M is sufficient for activation. The addition of N-hydroxysuccinimide (NHS) or N-hydroxysulfoxosuccinimid (NHSS) [3] to the activation buffer (step 2 of this protocol) in a concentration of ca. 5 mM can improve the coupling efficiency in certain cases.

! *The beads should be resuspended after the separation of the supernatant as soon as possible, to avoid decreasing binding capacities by drying.*

Literature:

- [1] A. Williams, I. A. Ibrahim, J. Am. Chem. Soc. 103 (1981) 7090.
- [2] M. A. Gilles, A. Q. Hudson, C. L. Borders, Jr., Anal. Biochem. 184 (1990) 244.
- [3] J. V. Staros, R. W. Wright, D. M. Swingle, Anal. Biochem. 156 (1986) 220.

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