

A typical protocol for Miltenyi MACS Beads
(uMACS Epitope Tag Protein Isolation Kits)

Plants (0.5 g)

- ↓ add appropriate Lysis Buffer (1.5 ml) (see below)
- ↓ homogenize with mortar/pestle
- ↓ centrifuge for 10 min at 10,000 rpm at 4°C
sup (1 ml)
- ↓ add 50 ul MicroBeads and mix well
- ↓ incubate on-ice for 10-30 min

u Column

- ↓ place column in the magnetic field of the uMACS Separator
- ↓ pre-wash with 200 ul of 0.1 M Na₂CO₃ (pH 11) pre-heated to 75°C
- ↓ equilibrate with 1 ml of Lysis Buffer
- ↓ apply the sample to column
- ↓ wash column with 4x200 ul of Lysis Buffer
- ↓ wash column with 100 ul of Wash Buffer (see below)
- ↓ apply 20 ul of 0.1 M Na₂CO₃ pre-heated to 75°C
- ↓ incubate for 5 min at room temperature
- ↓ elute with 50 ul of 0.1 M Na₂CO₃ pre-heated to 75°C
eluent
- ↓ add 5 ul of 1 M HCl or 10 ul of 1 M Mes for neutralization
sample for MS analysis

An example of Lysis Buffer

- 50 mM Tris-HCl (pH 8.0)
- 150 mM NaCl
- 1 mM CaCl₂
- 1 mM MgCl₂
- 1% CHAPS
- protease inhibitor (Roche complete EDTA-free)

Wash Buffer

- Lysis Buffer without salt and/or detergent

For SDS-PAGE analysis,

- use conventional SDS-PAGE Sample Buffer instead of 0.1 M Na₂CO₃.
- omit pre-wash step.