

iCapMag™

- IMPURITY
 - PROTEIN-TAG
 - ⊖ iCapMag™
 - PROTEIN NO TAG
- ⊖ Equilibrate pH

HOW THE TECHNOLOGY WORKS?



ADD ACTIVATED
MAGNETIC BEADS &
CLARIFIED CELL
LYSATE
pH 8.5

MIX & BIND
(est. incubation time
10 min to 2 hours)
pH 8.5

WASH 3-5x
(until UV-VIS
absorbance reaches
zero)
pH 8.5

SHIFT pH,
WAIT & CLEAVE
TARGET PROTEIN,
ELUTE
(change pH from
pH 8.5 to 6.2)

TRANSFER ELUENT
(purified & tagless
protein)

Binding Buffer pH 8.5

Wash pH 8.5

Elution Buffer pH 6.2

TEMPERATURE CONTROL

~4 °C (ice/fridge)

37 °C

20-25 °C

HIGHLY CONTROLLED SUPPRESSION OF CLEAVING

FASTER CLEAVING

OR
20-25 °C

STANDARD METHOD

iCapMag™

KEY BUFFERS

Activation Buffer: 6 M guanidine HCl and 0.5 M NaCl

pH 8.5 Binding Buffer:

20 mM AMPD

20 mM PIPES

200 mM NaCl

Adjust to pH 8.5 with 2M or 10M NaOH

pH 8.5 Wash Buffer:

20 mM AMPD

20 mM PIPES

500 mM NaCl

Adjust to pH 8.5 with 2M or 10M NaOH

pH 6.2 Elution Buffer:

20 mM AMPD

20 mM PIPES

200 mM NaCl

Adjust to pH 6.2 with 2M or 10M NaOH

*For more concentrated target protein use 2x BV

Storage Buffer: 20% ethanol, 2-8 °C

To store magnetic beads follow steps

“Preparation of Magnetic Beads” and

“Activation of Magnetic Beads” before

adding 20% ethanol.

Do not freeze or dry out the beads.

KEY STEPS

Preparation of Magnetic Beads:

Remove storage solution, wash beads (e.g., 100 µL bed volume) with binding buffer 2-3x (pH 8.5, 10xBVs) and rinse with water (10xBVs). Next, use magnetic rack to separate magnetic beads from the buffer and remove the buffer.

Activation of Magnetic Beads:

Add 6 M guanidine HCl and 0.5 M NaCl (10xBVs) & wait

Contact time est. 15-30 min

Next, wash beads 3x with binding buffer pH 8.5 (10xBVs)

Preparation of the Sample:

Prepare 5 mL of CCL in cold binding buffer pH 8.5. Filter CLL using 0.45 µm filter and 5 mL syringe to remove any cell debris. Take a sample (15 µL/tube) for SDS-PAGE.

Bind at pH 8.5

Add 1 mL of CCL containing **target protein-tag complex** to 100 µL of magnetic beads. Incubate it for 15 min. at ~4 °C (prolong incubation time up to 2h if needed; use ice or fridge). Next, use magnetic rack to separate magnetic beads from the buffer. Remove buffer containing non-bound protein-tag complex. Take a sample (15 µL/tube) for SDS-PAGE.

Wash at pH 8.5

Wash beads 3-5 x 1 mL (or 10xBVs) with cold wash buffer pH 8.5 until UV-VIS absorbance reaches zero. Use magnetic rack to separate magnetic beads from the buffer and remove the buffer. Take samples after 1st and last wash (15 µL/tube) for SDS-PAGE.

Elute at pH 6.2

Add 200 µL of pre-warmed elution buffer pH 6.2 (37 °C) to 100 µL of iCapMag™ beads. For faster cleavage incubate the sample at 37 °C (use either incubator, heating block or water bath). Take 3-4 samples (15 µL/tube) every 10-15 min. for SDS-PAGE. Each time register protein concentrations using UV-Vis at 280 nm. Follow steps to store magnetic beads.

ABBREVIATIONS:

AMPD: 2-amino-2-methyl-1,3-propanediol

PIPES: 1,4-piperazinediethanesulfonic acid

CCL: clarified cell lysate

CFS: collect flowthrough samples for analysis

BV: Bed volume(s) refers to approximate amount of magnetic beads slurry