



QuickPick™ CM

62201 • weak cation exchange kit for proteins, 8 preps

62211 • weak cation exchange kit for proteins, 48 preps

INTRODUCTION

These are the instructions for use for the QuickPick™ CM kits. Please read the instructions carefully before starting to work with the reagents. The QuickPick CM reagents are intended for use with the PickPen® magnetic tool and they provide a fast and simple means for protein sample pretreatment based on weak cation exchange. Also refer to the PickPen® instructions for use.

SPECIFICATIONS

Vessel format:	1.5 ml microtubes
Capacity per reaction:	60 µg ± 6 µg (aprotinin)
Sample volume:	300-1000 µl
Elution volume:	20-100 µl, 50 µl recommended
Preparation time for one sample:	5 min

KIT CONTENTS

8 preps (Product Number 62201):

CM Magnetic Particles (0.8 ml):	CM magnetic particles in 20 % aqueous ethanol
CM Regeneration Buffer (3.5 ml):	Na-acetate buffer (pH 5.0), NaCl, 0.02 % NaN ₃
CM Wash Buffer (15 ml):	Na-acetate buffer (pH 5.0), 0.02 % NaN ₃
CM Elution Buffer (0.8 ml):	100 mM TAPS buffer (pH 8.0), 300 mM NaCl, 0.02 % NaN ₃
PickPen [®] tips (1 x 8-Pack)	Ready to use

48 preps (Product Number 62211):

CM Magnetic Particles (5.0 ml):	CM magnetic particles in 20 % aqueous ethanol
CM Regeneration Buffer (20 ml):	Na-acetate buffer (pH 5.0), NaCl, 0.02 % NaN ₃
CM Wash Buffer (3 x 30 ml):	Na-acetate buffer (pH 5.0), 0.02 % NaN ₃
CM Elution Buffer (5.0 ml):	100 mM TAPS buffer (pH 8.0), 300 mM NaCl, 0.02 % NaN ₃
PickPen [®] tips (6 x 8-Pack)	Ready to use

ADDITIONAL MATERIAL REQUIRED BUT NOT SUPPLIED WITH THE KIT

1. PickPen[®] 1-M: The reagents are designed for use with the PickPen[®] magnetic tool, supplied by Bio-Nobile Oy. See also the PickPen[®] instructions for the use.
2. Microtubes or other suitable reaction tubes.

PRINCIPLE OF METHOD

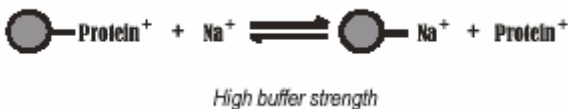
The weak cation exchange method in the Bio-Nobile QuickPick CM kit is based on magnetic cellulose particles coated with carboxymethyl (CM) groups. Different types of proteins will bind to the CM coated magnetic particles with affinities that depend on both the conditions used and the types and number of individual charged groups. Typically a mixture of numerous proteins, for example cell lysate, is used as a sample with ion exchangers. After unbound molecules are washed away, the composition of the buffer is changed by altering the pH and salt concentration to release the molecule(s) of interest from the particles.

Proteins consist of many different amino acids, and the overall net charge is caused by the composite effect of many different ionizable groups. The pH at which the protein has no net charge is called the isoelectric point, and is termed pI. The pI of most proteins is in the range of 5-9. Ion-exchange of proteins is typically performed at least 1 pH unit away from the pI of the protein of interest. **When the pH is below the pI, the molecule will be positively charged and a cation exchange resin should be used. When the pH is above the pI, the molecule will be negatively charged and an anion exchange resin should be used.** Since interactions of ion-exchange groups with proteins depend on the surface charges of the protein, even a protein at its pI may bind to the ion exchange matrix.

In the purification of proteins, a low buffer concentration must be used during binding. Otherwise the buffer components will compete with the proteins for the exchange sites.



If the concentration of the buffer ions is high the buffer ions will tend to bind to the ionic sites on the particles and any bound target proteins will tend to elute.



PROCEDURE

PickPen® tips

The PickPen® tips in the 8-Pack are ready to use. The tips can be autoclaved (+121 °C at least 20 min) or baked (+180 °C overnight) provided that they are first removed from the pack. The separately available PickPen® tip box can also be autoclaved.

The following notes are important for the procedure

1. Mix the CM Magnetic Particles suspension thoroughly before pipetting into microtubes.
2. Mild pulse vortexing may be used in the sample binding step to suspend the magnetic particles thoroughly in the sample.
3. The volume of the sample used may be between 300-1000 µl, and concentrated samples may be diluted using CM Wash Buffer as needed.
4. The volume of CM Elution Buffer may be between 20-100 µl depending on the protein concentration required for the downstream application.
5. If parallel reactions are performed simultaneously the next reaction may be started while the first sample is being incubated with magnetic particles in the sample tube. The PickPen® tip of the first reaction can be stored in an extra tube during the preparation of the next reaction. Remember to mix the sample tube of the first reaction occasionally.

Sample preparation

It is possible to use the QuickPick CM kit for a wide variety of sample preparations where separation of proteins based on weak cation exchange is needed. CM Wash Buffer is recommended as the sample buffer, however, any buffer with low ionic strength (<20 mM) and suitable pH can be used. Add protease inhibitors, protein stabilizers or other additives, if needed.

Sample preparation from bacterial and yeast cells or cultivated tissue cells

The most commonly used methods for disruption of cells are ultrasonication and French press. Bead mills are used for tough-to-disrupt cells like yeast.

Example 1:

Suspend *E. coli* cells (from 5-10 ml fermentation) or cultivated tissue cells ($0.5-2 \times 10^7$) into 1-3 ml CM Wash Buffer. Add protease inhibitors, protein stabilizers or other additives, if needed. Sonicate the suspension on an ice-bath with 10 x 5 s pulses. Leave a 10 s interval without sonication between each pulse to prevent warming of the suspension. After sonication, centrifuge the suspension at 10000 x g for 5 minutes and use the supernatant as the sample.

Example 2:

Suspend yeast cells (300-500 mg) in 1-2 ml of CM Wash Buffer. Add protease inhibitors, protein stabilizers or other additives, if needed. Pipette 1 ml of yeast suspension into a 1.5 ml tube, add glass beads to 1/3 volume of the tube and mix for 5 minutes in a bead mill. After cell disruption, centrifuge the suspension at 10000 x g for 5 minutes and use the supernatant as the sample.

Sample preparation from animal/plant tissues

There are various methods for mincing and homogenizing tissues, e.g. (i) grinding tissue in liquid nitrogen, (ii) homogenizers, (iii) sonicators and (iv) bead mills. As an example the sample preparation based on liquid nitrogen is described.

Pulverize frozen plant tissue or animal tissue in liquid nitrogen. Weigh the tissue while frozen. Suspend the pulverized material (200-250 mg plant or animal tissue) in 1-2 ml of CM Wash Buffer. Add protease inhibitors, protein stabilizers or other additives, if needed. Centrifuge the suspension at 10000 x g for 5 minutes and use the supernatant as the sample.

NOTE: The samples prepared according to these instructions can be further diluted using CM Wash Buffer as needed.

QuickPick™ CM kit protocol with PickPen® 1-M

Number six tubes from 1 to 6 and pipette liquids into tubes before starting as follows:

Tube 1: 100 µl CM Magnetic Particles in storage solution

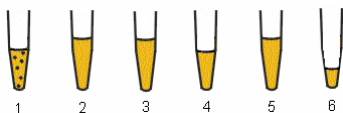
Tube 2: 400 µl CM Regeneration Buffer

Tube 3: 400 µl CM Wash Buffer

Tube 4: 300 µl sample

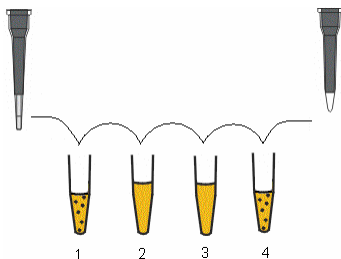
Tube 5: 400 µl CM Wash Buffer

Tube 6: 50 µl CM Elution Buffer



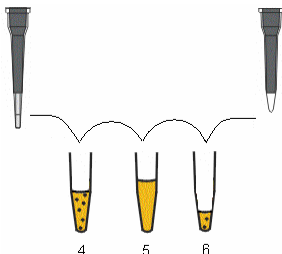
Protocol

1. Pick up the PickPen® tip from the tip pack using the PickPen® 1-M. Extend the magnet 2-3 times to check that the tip is firmly in place.
2. Collect the magnetic particles from tube 1 (CM Magnetic Particles) with PickPen®. Regenerate the magnetic particles by releasing into tube 2 (CM Regeneration Buffer). Mix the suspension in tube 2 with the PickPen® tip for 10 seconds. Note that the magnet has to be withdrawn at this point.
3. Collect the magnetic particles from tube 2 and release them into tube 3 (CM Wash Buffer). At this point the magnetic particles might be clumped together. The PickPen® tip (magnet withdrawn) can be used to break up the clump against the wall of the tube.
4. Collect the magnetic particles from tube 3 and release them into tube 4 (sample). Mix the magnetic particles thoroughly in the solution and incubate for at least 2 minutes. Mix the solution occasionally to avoid sedimentation.



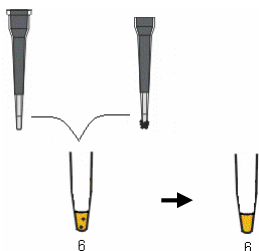
Incubate for at least 2 minutes

5. Collect the magnetic particles from tube 4 and wash them in tube 5 (CM Wash Buffer) by releasing the particles into solution. Mix the suspension in tube 5 with the PickPen[®] tip for 10 seconds. Note that the magnet has to be withdrawn at this point.
6. Collect the magnetic particles from tube 5 and release them into tube 6 (CM Elution Buffer) and mix thoroughly. Incubate for at least 1 minute. Mix the solution occasionally to avoid sedimentation.



Incubate for at least 1 minute

7. After incubation collect the magnetic particles from tube 6 and discard. The sample is now in the eluate and ready to be used for downstream applications.



STORAGE AND STABILITY

The QuickPick CM kit should be stored at +2°-+8°C. Magnetic particles should not be frozen.

WARNINGS AND LIMITATIONS

The QuickPick CM kit is intended for research use only, and not intended for use in human diagnostic or therapeutic procedures. All solutions except CM Magnetic Particles contain 0.02 % sodium azide (NaN₃) as a preservative. When in contact with acid or heavy metal ions, it forms highly toxic gas. Preservatives such as NaN₃ are toxic if ingested. Do not pipet by mouth. Direct skin contact must be avoided. Appropriate precautions should be taken when handling these solutions.

DISCLAIMERS AND WARRANTIES

Bio-Nobile warrants that its products shall be free from defects in materials and workmanship and shall meet performance specifications if stored and used in accordance with the instructions for use, for a period up to the expiry date provided on the kit package. This warranty does not cover normal wear and tear or misuse of the product. Bio-Nobile's obligation and the purchaser's exclusive remedy under this warranty is limited to replacement, at Bio-Nobile's expense, of any products defective in manufacture. In no event shall Bio-Nobile be liable for any special, incidental or consequential damages. This warranty statement may be subject to modification in accordance with local laws, regulations and business practices.

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BIO-NOBILE

Innovations for magnetic bioseparations

Bio-Nobile Oy,
Tykistökatu 4 B, P.O Box 36, FIN-20521 Turku, Finland
tel +358 2 410 1100
www.bio-nobile.com, info@bio-nobile.com

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