

QuickPick™ DEAE

62101 • weak anion exchange kit for proteins, 8 preps
 62111 • weak anion exchange kit for proteins, 48 preps

INTRODUCTION

These are the instructions for use for the QuickPick™ DEAE kits. Please read the instructions carefully before starting to work with the reagents. The QuickPick DEAE 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 anion exchange. Also refer to the PickPen® instructions for use.

SPECIFICATIONS

Vessel format:	1.5 ml microtubes
Capacity per reaction:	60 µg ± 6 µg (BSA)
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 62101):

DEAE Magnetic Particles (0.8 ml):	DEAE magnetic particles in 20 % aqueous ethanol with Tween 20
DEAE Regeneration Buffer (3.5 ml):	TAPS buffer (pH 8.0), containing NaCl, Tween 20, 0.02 % NaN ₃
DEAE Wash Buffer (15 ml):	TAPS buffer (pH 8.0), containing NaCl, Tween 20, 0.02 % NaN ₃
DEAE Elution Buffer (0.8 ml):	20 mM Na-acetate (pH 5.0), 300 mM NaCl, 0.05 % Tween 20, 0.02 % NaN ₃
PickPen® tips (1 x 8-Pack)	Ready to use

48 preps (Product Number 62111):

DEAE Magnetic Particles (5.0 ml):	DEAE magnetic particles in 20 % aqueous ethanol with Tween 20
DEAE Regeneration Buffer (20 ml):	TAPS buffer (pH 8.0), containing NaCl, Tween 20, 0.02 % NaN ₃
DEAE Wash Buffer (3 x 30 ml):	TAPS buffer (pH 8.0), containing NaCl, Tween 20, 0.02 % NaN ₃
DEAE Elution Buffer (5.0 ml):	20 mM Na-acetate (pH 5.0), 300 mM NaCl, 0.05 % Tween 20, 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 BN Products & Services Oy. See also the PickPen® instructions for the use.
2. Microtubes or other suitable reaction tubes.

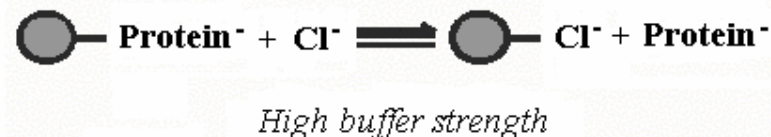
PRINCIPLE OF METHOD

The weak anion exchange method in the Bio-Nobile™ QuickPick™ DEAE kit is based on magnetic cellulose particles coated with diethylaminoethyl (DEAE) groups. Different types of proteins will bind to the DEAE 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 bound target proteins will tend to elute:



PROCEDURE

PickPen® tips

The PickPen® tips in the 8Pack 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. To avoid foaming, only mild pulse vortexing is recommended for all solutions (contain Tween 20).
2. Mix the DEAE Magnetic Particles suspension thoroughly before pipetting into reaction tubes.
3. Mild pulse vortexing may be used in the sample binding step to suspend the magnetic particles thoroughly in the sample.
4. The volume of the sample used may be between 300-1000 µl, and concentrated samples may be diluted using DEAE Wash Buffer as needed.
5. The volume of DEAE Elution Buffer may be between 20-100 µl depending on the protein concentration required for the downstream application.
6. 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 DEAE kit for a wide variety of sample preparations where separation of proteins based on weak anion exchange is relevant. DEAE Wash Buffer is recommended as the sample buffer, however, any buffer with low ionic strength (<20mM) 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 DEAE 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 DEAE Wash Buffer. Add protease inhibitors, protein stabilizers or other additives, if needed. Pipette 1 ml 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.

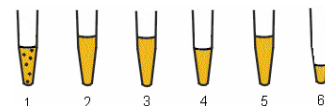
Pulverize frozen plant tissue or animal tissue in liquid nitrogen. Weigh the tissue while frozen. Suspend the pulverized material (200-250 mg plant or animal tissue) in 1-2 ml of DEAE Wash Buffer. Add protease inhibitors, protein stabilizers or other additives, if needed. Centrifuge the suspension at 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 DEAE Wash Buffer as needed.

QuickPick™ DEAE kit protocol with PickPen® 1-M

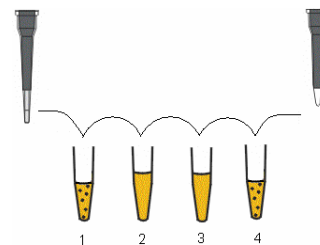
Number six tubes from 1 to 6 and pipette liquids into tubes before starting as follows:

- Tube 1: 100 µl DEAE Magnetic Particles in storage solution
- Tube 2: 400 µl DEAE Regeneration Buffer
- Tube 3: 400 µl DEAE Wash Buffer
- Tube 4: 300 µl sample
- Tube 5: 400 µl DEAE Wash Buffer
- Tube 6: 50 µl DEAE Elution Buffer



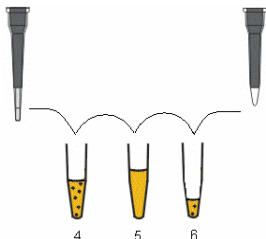
Protocol

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 (DEAE Magnetic Particles) with PickPen®. Regenerate the magnetic particles by releasing into tube 2 (DEAE 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 (DEAE Wash Buffer). The magnetic particles might be clumped together. Use the PickPen® tip (magnet withdrawn) 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.



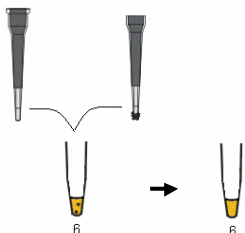
Incubate for at least 2 minutes

- Collect the magnetic particles from tube 4 and wash them in tube 5 (DEAE 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.
- Collect the magnetic particles from tube 5 and release them into tube 6 (DEAE Elution Buffer) and mix thoroughly. Incubate for at least 1 minute. Mix the solution occasionally to avoid sedimentation.



Incubate for at least 1 minute

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

WARNINGS AND LIMITATIONS

The QuickPick DEAE kit is intended for research use only, and not intended for use in human diagnostic or therapeutic procedures. All solutions except the DEAE Magnetic Particles contain 0.02 % sodium azide (NaN₃) as a preservative. When in contact with acid or heavy metal ions, it forms a 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.

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