

QuickPick™ GST kit

62401 • glutathione affinity kit for proteins, 8 preps

62411 • glutathione affinity kit for proteins, 48 preps

INTRODUCTION

These are the instructions for use for the QuickPick™ GST kits. Please read the instructions carefully before starting to work with the reagents. The QuickPick GST reagents are intended for use with the QuicPick magnetic tool and they provide a fast and simple means for recombinant Glutathione-S-transferase (GST) fusion protein purification. Also refer to the QuicPick instructions for use.

SPECIFICATIONS

Vessel format:	1.5 or 2.0 ml microtubes
Sample volume:	Cells from 3 - 10 ml of culture medium are suspended in 0.8 ml of Wash Buffer. The amount of culture required depends on the level at which the protein is expressed.
Elution volume:	100 µl
Capacity per reaction:	Up to 40 µg (Glutathione-S-transferase)
Total protocol time:	~ 12 min

KIT CONTENTS

62401

62411

GST Magnetic Particles	3.4 ml	20 ml
GST ^{10x} Wash Stock Buffer	3.0 ml	16 ml
GST Elution Mix Buffer	0.9 ml	5.6 ml
Glutathione, lyophilized	11 mg	69 mg
8-Pack QuicPick tips	1 pack	6 packs

GST Magnetic Particles:	GST Magnetic Particles in PBS buffer, Tween 20, 0.02 % NaN ₃
GST ^{10x} Wash Stock Buffer*:	After dilution to GST Wash Buffer: PBS (pH 7.4): Tween 20, 0.02 % NaN ₃
GST Elution Mix Buffer**:	100 mM Tris-HCl (pH 8.0), 0.05 % Tween 20, 0.02 % NaN ₃
Reduced Glutathione**:	glutathione, lyophilized powder

* Used for the preparation of GST Wash Buffer. The GST ^{10x}Wash Stock Buffer is a concentrated stock solution and has to be diluted before use.

** Used for the preparation of GST Elution Buffer.

ADDITIONAL MATERIAL REQUIRED BUT NOT SUPPLIED WITH THE KIT

1. QuicPick 1-M magnetic tool. See also the QuicPick 1-M instructions for use.
2. Microtubes or other suitable reaction tubes.

PRINCIPLE OF THE METHOD

QuickPick™ GST Magnetic Particles are formed from magnetic agarose particles which provide an extensive porous surface area especially suitable for the purification of recombinant GST-fusion proteins from cell lysates. The transfer of magnetic particles with QuicPick technology enables rapid purification of recombinant GST-fusion proteins. The methodology removes the need of using time-consuming column technology.

Glutathione-S-transferase (GST) is one of the most common affinity methods used to simplify the purification and detection of recombinant proteins expressed in *Escherichia coli* (*E. coli*). The principle of the mechanism is based on the interaction between a glutathione molecule covalently bound to magnetic particles via a spacer arm and a recombinant GST-fusion protein. An affinity elution using reduced glutathione liberates the GST-fusion proteins from the magnetic particles.

PROCEDURE

QuicPick tips

The QuicPick tips in the 8-Pack are sterile and ready to use. The tips packed in bulk quantities in plastic bags are not sterile, but can be autoclaved (+121 °C at least 20 min) provided that they are first removed from the bag. The separately available QuicPick tip box can also be autoclaved.

Note: Tips should be picked up gently from the pack. Too much pressure may open the pack.

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 GST Magnetic Particles suspension thoroughly before pipetting into reaction tubes.
3. Mild pulse vortexing may be used in sample binding step to suspend the magnetic particles thoroughly in the sample.
4. Between 500 and 1000 µl of resuspended cell material can be used as sample.
5. The volume of GST Elution Buffer may be between 70-100 µl depending on the protein concentration required for the downstream application.
6. If parallel reactions are performed simultaneously the second reaction may be started while the first sample is being incubated with magnetic particles in the sample tube. The QuicPick tip of the first reaction can be stored in extra tube during the preparation of the second reaction. Remember to mix the sample tube of the first reaction occasionally.

Sample preparation

It is possible to use the QuickPick GST kit for a wide variety of sample preparations, where purification of GST-fusion proteins is needed. GST Wash Buffer made from GST ^{10x}Wash Stock Buffer is recommended as the sample buffer. Add protease inhibitors, protein stabilizers or other additives, if needed.

The most commonly used methods for disruption of bacterial cells are ultrasonication and French press.

Example: Suspend *E. coli* cells (from 3-10 ml fermentation) into 0.8 ml GST Wash Buffer. Add protease inhibitors, protein stabilizers or other additives, if needed. Sonicate the suspension on an ice-bath with 10 x 10 s pulses. Leave a 10 s interval without sonication between each pulse to prevent warming of the suspension. After sonication, centrifuge the suspension 10 minutes at 18 000 x g and use the supernatant as the sample. (If lower centrifugation speed is used the centrifugation time should be increased, for example 20 minutes at 10 000 x g).

Preparing GST Wash Buffer from GST ^{10x}Wash Stock Buffer

Prepare by diluting GST ^{10x}Wash Stock Buffer in distilled water as follows:

For 8 preparations (kit 62401) pipet:

	3 ml GST ^{10x} Wash Stock Buffer
	<u>27 ml</u> H ₂ O
Total:	30 ml

For 48 preparations (kit 62411) pipet:

	16 ml GST ^{10x} Wash Stock Buffer
	<u>144 ml</u> H ₂ O
Total:	160 ml

The GST Wash Buffer should be stored at +2°-+8°C.

Preparing the GST Elution Buffer from the lyophilized glutathione and the GST Elution Mix Buffer

Prepare GST Elution Buffer containing 40 mM reduced glutathione as follows:

Open the lyophilized Glutathione vial carefully to avoid release of powder and pipet GST Elution Mix Buffer into the vial.

For **8 preparations** kit (62401) pipet **0.9 ml** GST Elution Mix Buffer
For **48 preparations** kit (62411) pipet **5.6 ml** GST Elution Mix Buffer

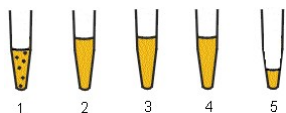
Once the GST Elution Buffer is prepared, it must be divided into aliquots and stored at -20°C to prevent oxidation of the glutathione.

QuickPick™ GST protocol with QuicPick

All solutions should be clear when used. If precipitates have formed warm the solutions gently until the precipitates have dissolved. Make sure that GST Wash Buffer is correctly prepared from the GST ^{10x}Wash Stock Buffer before continuing. GST Magnetic Particles should be mixed thoroughly just before pipetting. Repeat pipettors should not be used when dispensing magnetic particles.

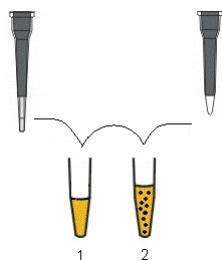
Number tubes from 1 to 5 and pipette liquids before starting as follows:

- Tube 1: 400 µl GST Magnetic Particles
- Tube 2: 800 µl sample (see: “**Sample preparation**”)
- Tube 3: 800 µl GST Wash Buffer
- Tube 4: 800 µl GST Wash Buffer
- Tube 5: 100 µl GST Elution Buffer



Protocol

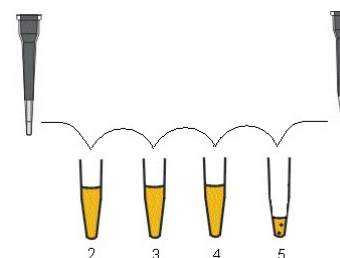
1. Pick up the QuicPick tip using the QuicPick. Collect the magnetic particles from tube 1 (Magnetic Particles) and release them into tube 2 (sample). If the magnetic particles have formed clumps, they can be broken up by using the QuicPick tip. Mix briefly and gently. Note that the magnet has to be withdrawn at this point. Incubate for at least 5 minutes. Mix the solution occasionally to avoid sedimentation.



Incubate for at least 5 minutes

2. Collect the magnetic particles from tube 2 and wash them in tube 3 (Wash Buffer) by releasing the magnetic particles into the solution and mixing gently. Repeat the washing in tube 4. Collect the particles from tube 4 (start collecting the magnetic particles from the very surface of the liquid to ensure

that they collect only onto the end of tip) and release carefully into tube 5 (Elution Buffer). Rub the tip against the tube wall to ensure that all magnetic particles are released into the suspension. Mix the magnetic particles thoroughly but gently in the solution and incubate for at least 5 minutes. Mix the solution occasionally to avoid sedimentation.



Incubate for at least 5 minutes

4. After incubation collect the magnetic particles from tube 5 and discard. The purified GST-tagged protein is ready to be used for downstream applications.



Collect the magnetic particles and discard them.

STORAGE AND STABILITY

The QuickPick GST kit should be stored at +2°-+8°C. Magnetic particles should not be frozen. Once the GST Elution Buffer is prepared, it must be divided into working volumes and stored at -20°C to prevent oxidation of the glutathione.

WARNINGS AND LIMITATIONS

The QuickPick GST kit is intended for research use only, and not intended for use in human diagnostic or therapeutic procedures.

All solutions contain 0.02-0.04 % 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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