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About the Kits

RoboPop Ni-NTA His•Bind Purification Kit	71188-3
RoboPop GST•Bind Purification Kit	71189-3

Description

The RoboPop Purification Kits are designed for high-throughput (HT) purification of His•Tag® and GST•Tag™ fusion proteins directly from *E. coli* cultures without cell harvest, mechanical disruption, or extract clarification. The kits feature PopCulture® Reagent, rLysozyme™ Solution, Benzonase® Nuclease, Ni-NTA His•Bind or GST•Bind Resin, and buffers for efficient protein extraction and affinity purification. These RoboPop Purification Kits are designed to purify recombinant fusion protein from 96 × 5 ml cultures using a 2-ml well capacity filter plate. The 96-Well Filter Plate is compatible with standard filter manifolds for manual and robotic processing. Automated processing is validated on the MultiPROBE® II HT EX liquid handling workstation (from PerkinElmer Life Sciences) and the Genesis Freedom™ Workstation (Tecan). A 96-Well Collection Plate (1.0-ml wells) and Sealer is provided for storage of the purified proteins. The RoboPop Ni-NTA His•Bind Purification Kit will purify up to 96 mg His•Tag fusion proteins per plate (up to 1 mg/well) and the RoboPop GST•Bind Purification Kit will purify up to 77 mg GST•Tag fusion proteins per plate (up to 0.8 mg/well). Stated yields are based on 5-ml cultures and binding capacities of the resin, and vary with expression levels, folding properties, and solubilities for individual fusion proteins.

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Components

RoboPop Ni-NTA His•Bind Purification Kit

- 75 ml PopCulture® Reagent
- 1 2 ml 96-Well Filter Plate
- 1 1 ml 96-Well Collection Plate with Sealer
- 25 ml Ni-NTA His•Bind Resin, 25 ml settled volume (50 ml of a 50% v/v suspension)
- 125 ml 4X Ni-NTA Bind Buffer (4X = 1.2 M NaCl, 200 mM Na phosphate, 40 mM imidazole, pH 8.0)
- 2 × 125 ml 4X Ni-NTA His•Bind Wash Buffer (4X = 1.2 M NaCl, 200 mM Na phosphate, 80 mM imidazole, pH 8.0)
- 50 ml 4X Ni-NTA His•Bind Elute Buffer (4X = 1.2 M NaCl, 1.0 M imidazole, 200 mM Na Phosphate, pH 8.0)
- 300 KU rLysozyme™ Solution (30 KU/µl in 100 mM NaCl, 50 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50% glycerol, 0.1% Triton® X-100, pH 7.5)
- 1 ml rLysozyme Dilution Buffer (100 mM NaCl, 50 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 0.1% Triton® X-100, pH 7.5)
- 10 KU Benzonase® Nuclease, Purity > 90%

RoboPop GST•Bind Purification Kit

- 75 ml PopCulture Reagent
- 1 2 ml 96-Well Filter Plate
- 1 1 ml 96-Well Collection Plate with Sealer
- 25 ml GST•Bind Resin, 25 ml settled volume (50 ml of a 50% v/v suspension)
- 100 ml 10X GST•Bind/Wash Buffer (10X = 1.37 M NaCl, 43 mM Na_2HPO_4, 27 mM KCl, 14.7 mM KH_2PO_4, pH 7.3)
- 40 ml 10X Glutathione Reconstitution Buffer (10X = 500 mM Tris-HCl, pH 8.0)
- 1 g Glutathione, Reduced, Free Acid
- 300 KU rLysozyme Solution (30 KU/µl in 100 mM NaCl, 50 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50% glycerol, 0.1% Triton® X-100, pH 7.5)
- 1 ml rLysozyme Dilution Buffer (100 mM NaCl, 50 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 0.1% Triton® X-100, pH 7.5)
- 10 KU Benzonase Nuclease, Purity > 90%

Storage

Store PopCulture Reagent, Sterile 96-Well Collection Plate and Sealer and 96-Well Filter Plate at room temperature. Store Ni-NTA His•Bind Resin, 4X Ni-NTA Bind Buffer, 4X Ni-NTA Wash Buffer, 4X Ni-NTA Elute Buffer, GST•Bind Resin, 10X GST Bind/Wash Buffer, 10X Glutathione Reconstitution Buffer and Glutathione, Reduced, Free Acid at 4°C. Store Benzonase, rLysozyme Solution and rLysozyme Dilution Buffer at –20°C.

Notes: *Storage of PopCulture Reagent at or below 4°C may cause precipitation. Incubate PopCulture in a room temperature water bath with gentle swirling or inversion to redissolve.*

Diluted rLysozyme Solution should be stored at 4°C or on ice and used as soon as possible. Diluted rLysozyme should not be stored at –20°C because freezing may result in loss of activity.

DO NOT FREEZE the Ni-NTA His•Bind or GST•Bind Resin. Freezing results in complete loss of binding.

RoboPop Purification Kits

The general procedure is indicated below followed by detailed protocols in the subsequent sections. If manual processing is desired, use an 8- or 12-tip multichannel pipettor instead of programming a robotic liquid handling system. An optimized robotic program for the Packard-brand MultiPROBE® II HT EX using WinPrep® software can be downloaded from http://www.novagen.com/RoboPopsoftware. When using WinPrep software, the operator must establish Gripper™ positions for each instrument. For Tecan Genesis Freedom™ workstation software, please contact your Tecan Sales Representative.

Quick protocol: cell culture, extraction and purification

1. Culture cells in 3.0–5.0 ml × 96 wells using a 4 × 24-well culture plate (10 ml capacity) under conditions for target protein production. The sterile 24-well culture plates (10 ml capacity) with lids are available from VWR International (Cat. No. 13503-190). To avoid cross contamination between wells, use air-permeable BugStopper™ Venting capmats during cell culturing (VWR International, Cat. No. 14217-208)
2. Add 0.5 ml PopCulture® Reagent (0.1 ml/ml culture) containing 100 U (20 U/ml culture) Benzonase® Nuclease and 200 U (40 U/ml culture) rLysozyme™ Solution to each well. Mix and incubate 10 min at room temperature.
3. Optional: Take a sample from each well for screening expression levels using the following compatible protein assays including FRETWorks™ S•Tag™ Assay, SDS-PAGE, Western blot or other method.
4. Add 250 µl equilibrated Ni-NTA His•Bind or GST•Bind Resin (50 µl settled volume/ml culture). Incubate with mixing for 5 min at room temperature.
5. Transfer the mixture to the 96-Well Filter Plate and separate the resin from the extract with the vacuum manifold.
6. Wash the resin 2 times by resuspending in 2 ml (8 settled bed volumes) appropriate wash buffer. Remove the supernatant for each wash by applying a gentle vacuum.
7. Elute the target protein by resuspending the resin in 750 µl (3 settled bed volumes) appropriate elution buffer. Collect the filtrate containing the target protein into the 96-Well Collection Plate using the vacuum manifold.

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Cell culture

Induction using IPTG

These conditions may require optimization depending on the expression system, target protein, host strain, growth medium, temperature, and orbital-shaking incubator used. The following protocol is based on culturing Tuner™(DE3) cells. 5.0-ml cultures can be conveniently grown in 24-well deep well (10 ml) plates. Four 24-well plates of 5 ml cultures can be purified with the kit. Adjust culture size to avoid cross-contamination during shaking incubation.

1. Streak a glycerol stock or plate freshly transformed *E. coli* containing the expression plasmid and incubate overnight on LB agar plates containing appropriate antibiotics.
2. The following day, pipet 0.25 ml pre-warmed medium and appropriate antibiotics into each well of a 24-well culture plate (10 ml capacity). The 24-well culture plates (10 ml capacity) are available from VWR International (Cat. No. 13503-190). To avoid cross-contamination between wells, use air-permeable BugStopper™ Venting capmats during cell culturing (VWR International, Cat. No. 14217-208).

Note: Dispense the medium immediately prior to inoculation (step 3). Use pre-warmed at 24°C medium to avoid a lag phase in bacterial growth.

The initial 24°C growth temperature was chosen to grow the cells for 16 h without reaching saturation phase. The subsequent 30°C incubation may enhance solubility as compared to a 37°C incubation. These temperatures can be optimized for individual target proteins.

3. Using a sterile pipet tip, inoculate 1 colony into each well and swirl briefly to dislodge the colony into the medium.
4. Cover the inoculated plate with an air permeable sealer or lid.
5. Incubate at 24°C, shaking at 200 rpm for approximately 16 h.
6. Remove the sealer or lid and add an additional 4.75 ml pre-warmed (30°C) medium containing appropriate antibiotics into each well.
7. Reseal the culture plate.
8. Incubate at 30°C, shaking at 250 rpm approximately 1–2 h. Ideally the OD_{600} after 2–3 h incubation will be 1–1.5.

Note: To determine the OD, cut the membrane above a well or remove the lid and remove a sample from the well. Note that OD variation in the individual wells can occur.

9. Induce target protein expression with IPTG for approximately 3 h at 30°C. Use a concentration of IPTG appropriate for the expression vector. Optimize induction conditions as required for the expression system. The OD_{600} after induction in our experiments is typically 4–5. Final optical densities may vary.

Induction using Overnight Express™ Autoinduction System 1

The Overnight Express Autoinduction System 1 (Cat. No. 71300) is designed for high-level protein expression with the pET bacterial expression system without monitoring cell growth (1). In λDE3 lysogenic hosts, uninduced cells are grown to a high cell density followed by spontaneous induction of T7 RNA polymerase expression without monitoring cell density or adding IPTG. The Overnight Express Autoinduction System 1 is compatible with traditional glucose-free media and results in maximum yields of target proteins with the pET system (2). See Technical Bulletin 383 for more information.

The conditions described below may require optimization depending on the expression system, target protein, host strain, growth medium, temperature, culture volume and orbital-shaking incubator used. The following protocols is are based on culturing BL21(DE3) cells. 5.0-ml cultures can be conveniently grown in 24-well deep well (10 ml) plates. Four 24-well plates of 5 ml cultures can be purified with the kit. Adjust culture size to avoid cross-contamination during shaking incubation.

Note: Because lactose is used for induction, expression hosts should produce functional Lac permease (encoded by the lacY *gene) and β-galactosidase (encoded by the* lacZ *gene) for consistent results in both complex and defined media. Strains that are* lacY *mutants (i.e., the Tuner™, Origami™ B and Rosetta-gami™ B strains) or* lacZ *mutants should not be used. See "Additional Guidelines" for more information.*

1. Streak a glycerol stock or plate freshly transformed *E. coli* containing the expression plasmid and incubate overnight on LB agar plates containing appropriate antibiotics.
2. Prepare Overnight Express Autoinduction System 1 medium aseptically by combining sterile glucose-free medium with 0.02 vol OnEx™ Solution 1, 0.05 vol OnEx Solution 2, and 0.001 vol OnEx Solution 3 (see Table below). Add appropriate antibiotics for the host strain and plasmid.

Note: Overnight Express™ Autoinduction System 1 is compatible with glucose-free media such as Terrific Broth (TB), Luria-Bertani (LB) broth, and 2X YT. See "Additional Guidelines" for media recipes.

Overnight Express System 1 medium
Aseptically combine the following reagents
Per liter:
20 ml OnEx™ Solution 1
50 ml OnEx Solution 2
1 ml OnEx Solution 3
929 ml sterile glucose-free medium

3. The following day, pipet 5 ml pre-warmed Overnight Express System 1 medium plus appropriate antibiotics into each well of the a 24-well culture plate (10 ml capacity). The 24-well culture plates (10 ml capacity) are available from VWR International (Cat. No. 13503-190). To avoid cross-contamination between wells, use air-permeable BugStopper™ Venting capmats during cell culturing (VWR International, Cat. No. 14217-208).

Note: Dispense the medium immediately prior to inoculation (step 4). Use pre-warmed medium to avoid a lag phase in bacterial growth.

4. Using a sterile pipet tip, inoculate 1 colony into each well of the 24-well culture plate.
5. Cover the inoculated plate with an air permeable sealer or lid.
6. Incubate at 30°C, shaking at 200 rpm for approximately 16 hours.

Note: It is important to culture cells to stationary phase when using the Overnight Express System 1. See "Additional Guidelines" for more information.

PopCulture cell extract preparation

1. After induction is complete, transfer the 24-well culture plates (up to 4) to the robotic shaker platform.
2. Remove the culture plate sealer or lid.
3. Add 0.1 ml PopCulture® Reagent, 40 U (1 µl of a 1:750 dilution) rLysozyme™ Solution and 25 U (1 µl) Benzonase® Nuclease per ml original culture volume.
   a) The rLysozyme and Benzonase Nuclease can be pre-mixed with PopCulture Reagent for single-step addition. Pre-mixed PopCulture, rLysozyme Solution and Benzonase Nuclease (50 ml PopCulture Reagent, 20 KU rLysozyme, and 10 KU Benzonase per 96 × 5 ml cultures) should be stored until use at 4°C and used the same day.
   b) Addition of rLysozyme is not necessary if the host strain contains the pLysS or pLysE plasmid.
   c) rLysozyme Solution can be diluted with rLysozyme Dilution Buffer as necessary. Diluted rLysozyme Solution should be stored on ice or at 4°C and used the same day.
4. Incubate with gentle shaking for 10–15 min at room temperature.

Note: Protease Inhibitors may be added with the PopCulture Reagent if desired. EDTA is not compatible with Ni-NTA His•Bind Resin but is compatible with GST•Bind purification.

5. Optional: Remove a sample from each well for screening expression levels using compatible protein assays. The FRETWorks™ S•Tag™ Assay (Technical Bulletin 251), SDS-PAGE and Western blotting (His•Tag® Monoclonal Antibody, Cat. No. 70796-3) are compatible. The sample can be stored at 4°C until purification is complete.

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6. The prepared extract is combined directly with equilibrated GST•Bind or Ni-NTA His•Bind Resin for protein purification as described in the following sections.

Ni-NTA His•Bind purification

Note: In these protocols, one volume is defined as the settled resin volume (e.g. 100 µl of slurry yields 50 µl of resin). The resin volume used per 5 ml culture can be adjusted according to expression levels. However, generally 500 µl of slurry or 250 µl of settled resin is recommended per 5 ml culture.

Buffer preparation

The buffer volumes required will vary depending on the pipetting capabilities of the liquid handling instrument employed. To eliminate the possibility for insufficient buffer/reagent addition, a 20% reservoir overfill is recommended. Excess buffers are provided in the kit for this purpose. The following buffer volume recommendations do not include extra buffer for the reservoir overfill. Adjust according to the liquid handling capabilities.

1. Dilute an appropriate amount of the supplied stock of 4X Ni-NTA Bind Buffer to 1X with sterile deionized water. Approximately 260 ml of 1X Ni-NTA Bind Buffer is required for Ni-NTA His•Bind Resin preparation per 96-well plate
2. Dilute an appropriate amount of the supplied stock of 4X Ni-NTA Wash Buffer to 1X with sterile deionized water. Approximately 576 ml 1X Ni-NTA Wash Buffer is required per 96-well plate.
3. Dilute the 4X Ni-NTA Elute Buffer to 1X with sterile deionized water. Approximately 72 ml of 1X Ni-NTA Elute Buffer is required per 96-well plate.

Ni-NTA His•Bind Resin preparation

1. Gently mix the bottle of Ni-NTA His•Bind Resin by inversion until completely suspended. Transfer 25 ml resuspended slurry to each of two 50 ml Falcon tubes.
2. Centrifuge at 500 × g for 3 min to collect the resin.
3. Carefully remove the supernatant and wash the resin in each tube 3 times with 3 volumes (38 ml) 1X Ni-NTA Bind Buffer. For each wash, resuspend the resin with buffer, centrifuge at 500 × g for 3 min, and remove the supernatant carefully by pipetting.
4. After the final wash, resuspend each tube of resin using 1X Ni-NTA Bind Buffer for a final volume of 28 ml.
5. Dispense 3.5 ml portions of resuspended slurry (from both tubes) into a total of 16 (4 × 4) wells of a 24-well plate (10 ml capacity). This configuration facilitates pipetting the resin into the 24-well cultures during the following robotic purification procedure. The 24-well culture plates (10 ml capacity) are available from Whatman (Cat. No. 7701-5102).

Ni-NTA His•Bind purification

Purification is performed at room temperature. Allow the resin and buffers to equilibrate to room temperature before use.

Tip: Disposable pipet tips should be used where appropriate to prevent cross contamination between sample wells.

1. Mix the equilibrated resin slurry prepared in the previous section on a shaking platform. It is important that the slurry is evenly suspended. Pipetting may be necessary to get an even suspension just prior to aliquoting the resin (in the next step).
2. Add 0.5 ml the equilibrated Ni-NTA His•Bind Resin slurry to each well of prepared cell extract (5-ml cultures).
3. Mix thoroughly on a shaking platform for 5–10 min. Continue to mix the suspensions during transfer to the Filter Plate in step 4.
4. Place the 96-Well Filter Plate on the vacuum manifold and transfer the suspensions to the Filter Plate. Up to four 24-well culture extracts can be transferred to the 96-Well Filter Plate. Depending on the reaction volume and pipetting capabilities of the instrument, repeated transfers and intermittent vacuum application may be required.

5. Apply gentle vacuum to remove the supernatant. Separation time may vary depending on the type of manifold and vacuum parameters.
   Caution: Do not pull excess air through the resin bed after the filtrate has been removed. Drying of the resin bed can reduce yield and quality of the purified protein. Vacuum parameters should be adjusted to optimize the filtrate removal without resin bed drying.

Note: The supernatant could be retained for analysis of any target protein that did not bind to the resin if the filtrate is captured in a 96-well plate.

6. Wash the resin in each well 2–3 times with 8 volumes (2.0 ml) 1X Ni-NTA Wash Buffer. For each wash:
   a) Rapidly dispense the Ni-NTA Wash Buffer to resuspend the resin. Disposable pipet tips are not necessary during the wash procedure. Pipetting up and down
   b) Separate the resin from the wash buffer with vacuum application. Separation time may vary depending of the type of manifold and vacuum parameter.
   Caution: Do not pull excess air through the resin bed after the filtrate has been removed. Drying of the resin bed can reduce yield and quality of the purified protein. Vacuum parameters should be adjusted to optimize the filtrate removal without resin bed drying.
7. Elute the bound protein with 2–3 volumes (500–750 µl) 1X Ni-NTA Elute Buffer. For each elution:
   a) Position the 96-well Collection Plate on the robotic platform to capture the filtrate.
   b) Rapidly dispense the Ni-NTA Elute Buffer the resuspend the resin. Disposable pipet tips are not necessary during the elution procedure.
   c) Separate the Ni-NTA His•Bind Resin from the purified proteins in the Elute Buffer with vacuum application and collect into the 96-well Collection Plate.
8. Analyze the elution fraction from step 7. The prepared extract is compatible with Western blotting (His•Tag® Monoclonal Antibody, Cat. No. 70796-3), SDS-PAGE analysis, and other protein assays (e.g. FRETWorks™ S•Tag™ Assay, Technical Bulletin 251). The sample can be stored at 4°C until purification is complete.
   Optional: Analyze the supernatant from step 5 for the presence of the target protein. Detection can be performed by Western blotting with the His•Tag Monoclonal Antibody. Target protein that is insoluble or folded so that the His•Tag peptide is inaccessible will not bind to the Ni-NTA His•Bind Resin.
9. Store the eluted samples at 4°C. Storage conditions to retain activity are protein dependent. Apply the Collection Plate Sealer to prevent evaporation.

Instructions for the Collection Plate Sealer

The two-layer Collection Plate Sealer has positioning tabs at both ends. After the Sealer is fully secured on the culture plate, you can remove positioning tabs or layers on the tabs. Do not remove positioning tabs or layers from the tabs until the sealer is fully secured on the Collection Plate. The bottom layer of the Sealer is brown and when removed exposes the adhesive. The top layer is foil and covers the purified protein samples in the Collection Plate.

1. Remove the brown bottom layer to expose the adhesive. Use the positioning tabs for handling.
2. Position the Sealer on the Collection Plate.
3. Secure the Sealer by rubbing across the foil to ensure the adhesive is attached between each well.
4. To remove the Sealer from the plate, lift from a corner and gently pull.
5. To remove a sample from a well, cut the foil above the well with a razor blade to allow the pipet tip to reach the well. Sealers are single-use and after removal cannot re-seal the wells.

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RoboPop GST•Bind purification

Note: In these protocols, one volume is defined as the settled resin volume (e.g. 100 µl of slurry yields 50 µl of resin). The resin volume used per 5 ml culture can be adjusted according to expression levels. However, generally 500 µl of slurry or 250 µl of settled resin is recommended per 5 ml culture.

Buffer preparation

The buffer volumes required will vary depending on the pipetting capabilities of the liquid handling instrument employed. To eliminate the possibility for insufficient buffer/reagent addition, a 20% reservoir overfill is recommended. Excess buffers are provided in the kit for this purpose. The following buffer volume recommendations do not include extra buffer for the reservoir overfill. Adjust according to the liquid handling system.

1. Dilute an appropriate amount of the supplied stock of 10X GST•Bind/Wash Buffer to 1X with sterile deionized water. Approximately 840 ml 1X GST•Bind/Wash Buffer is required for GST•Bind Resin preparation and sample processing.
2. Prepare 10X GST Elution Buffer containing 100 mM Reduced Glutathione by dissolving 1 g Reduced Glutathione in 32.5 ml 10X Glutathione Reconstitution Buffer. After the 10X GST Elution Buffer is prepared, it must be divided into working volumes (75 ml per 96 well, 19 ml per 24 wells) and stored at –20°C to minimize oxidation of the glutathione. 10X GST Elution Buffer is stable at –20°C for 6 months with no more than 5 freeze/thaws. Immediately before use, dilute the 10X GST Elution Buffer to 1X with sterile deionized water. Approximately 75 ml 1X GST Elution Buffer is required per 96-well plate.

Note: The 1X GST Elution Buffer must be prepared from the 10X stock immediately before use.

GST•Bind Resin preparation

1. Gently mix the bottle of GST•Bind Resin by inversion until completely suspended. Transfer 25 ml resuspended slurry to each of two 50 ml Falcon tubes.
2. Centrifuge at 500 × g for 3 min to collect the resin.
3. Remove the supernatant and wash the resin in each tube 3 times with 3 volumes (38 ml) 1X GST Bind/Wash Buffer. For each wash, resuspend the resin with buffer, centrifuge at 500 × g for 3 min, and remove the supernatant carefully by pipetting.
4. After the final wash, resuspend the resin in each tube using1X GST Bind/Wash Buffer for a final volume of 28 ml.
5. Dispense 3.5 ml portions of resuspended slurry (from both tubes) into a total of 16 (4 × 4) wells of a 24-well plate (10 ml capacity). This configuration facilitates pipetting the resin into the 24-well cultures during the following robotic purification procedure. The 24-well culture plates (10 ml capacity) are available from Whatman (Cat. No. 7701-5102).

GST•Bind purification

Purification is performed at room temperature. Allow the resin and buffers to equilibrate to room temperature before use.

Tip: Disposable pipet tips should be used where appropriate to prevent cross contamination between sample wells.

1. Mix the equilibrated slurry prepared in the previous section on a shaking platform. It is important that the slurry is evenly suspended. Pipetting may be necessary to get an even suspension just prior to aliquoting the resin (in the next step).
2. Add 0.5 ml the equilibrated GST•Bind Resin slurry to each well of prepared cell extract (5-ml cultures).
3. Mix thoroughly on a shaking platform for 5–10 min. Continue to mix the resuspensions during transfer to the Filter Plate in step 4.
4. Place the 96-Well Filter Plate on the vacuum manifold and transfer the suspensions to the Filter Plate. Depending on the reaction volume and pipetting capabilities of the instrument repeated transfers and intermittent vacuum application might be required.

5. Apply gentle vacuum to remove the supernatant. Separation time may vary depending on the type of manifold and vacuum parameters.
   Caution: Do not pull excess air through the resin bed after the filtrate had been removed. Drying of the resin bed can reduce yield and quality of the purified protein. Vacuum parameters should be adjusted to optimize the filtrate removal without resin bed drying.

Note: The supernatant could be retained for analysis of any target protein that did not bind to the resin if the filtrate is captured in a 96-well plate.

6. Wash the resin in each well 2–3 times with 8 volumes (2.0 ml) 1X GST Bind/Wash Buffer. For each wash:
   a) Rapidly dispense the GST•Bind/Wash Buffer to resuspend the resin. Disposable pipette tips are not necessary during the wash procedure.
   b) Separate the GST•Bind Resin from the wash with vacuum application. Separation time may vary depending on the type of manifold and vacuum parameters.
   Caution: Do not pull excess air through the resin bed after the filtrate had been removed. Drying of the resin bed can reduce yield and quality of the purified protein. Vacuum parameters should be adjusted to optimize the filtrate removal without resin bed drying.
7. Elute the bound protein from the resin in each well with 2–3 volumes (500–750 µl) 1X GST Elution Buffer. For each elution:
   a) Position the 96-well Collection Plate on the robotic platform to capture the filtrate
   b) Rapidly dispense the GST Elute Buffer to resuspend the resin. Disposable pipet tips are not necessary during the elution procedure.
   c) Separate the GST•Bind Resin from the purified proteins with vacuum application into the Collection Plate.
8. Analyze the elution fraction from step 7. The GST•Tag™ Monoclonal Antibody (Cat. No. 71097-3) is available for Western analysis. Bradford and S•Tag™ FRETWorks™ or Rapid Assays are directly compatible with the eluted fraction. Because the GST Elution buffer is not compatible with Lowry protein assay, BCA Protein Assay Kit (Cat. No. 71285-3), and the GST•Tag Assay Kit (Cat. No. 70532-3), a buffer exchange must be done before these assays can be performed (See "Processing the Sample after Elution").
   Optional: Analyze the supernatant from step 5 for the presence of the target protein. Target protein that does not have functional GST and/or is insoluble will not bind to the GST•Bind Resin and can be detected by Western blotting or protein assays as indicated above (with the exception of the GST•Tag Assay).
9. Store the eluted samples at 4°C. Storage conditions to retain activity are protein dependent. Apply the Collection Plate Sealer to prevent evaporation. See page 8 for Collection Plate Sealer instructions.

Processing the Sample after Elution

The purified protein samples can be concentrated or the buffer changed by one of several methods. The storage buffer for your purified protein is often determined through an empirical process. Inappropriate storage buffer may lead to precipitation of the protein.

1. Dialyze into the buffer of choice. A 3-fold concentration can be achieved by dialysis into storage buffer containing 50% glycerol. Glycerol often stabilizes proteins for long-term storage. 96-well dialysis plates are available from Harvard Apparatus to facilitate processing.
2. Use 96-well microconcentrator units (e.g., Microcon-96 Filtrate Assemblies, Mililpore) or 96-well solid phase extraction plates (SPE Bioplates, Whatman) as directed by the manufacturer to concentrate the sample or perform additional rounds of purification.

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Additional Guidelines

Cell culture

Culture Medium: PopCulture® Reagent is compatible with Terrific Broth (TB; (3), Luria-Bertani (LB) and 2X YT media. TB promotes rapid growth and high cell densities (4).

LB: Per liter:

To 950 ml of deionized water add:

Bacto® tryptone	10 g
Bacto yeast extract	5 g
NaCl	10 g

Stir until dissolved. Adjust the pH to 7.0 with 5 N NaOH (~0.2 ml). Adjust the volume of the solution to 1 liter with deionized water and autoclave.

TB: Per liter:

To 900 ml of deionized water add:

Bacto tryptone	12 g
Bacto yeast extract	24 g
glycerol	4 ml

Stir until dissolved and sterilize by autoclaving for 20 min. Allow the solution to cool to < 60°C and then add 100 ml sterile 0.17 M KH_2PO_4, 0.72 M K_2HPO_4 solution (dissolve 2.31 g KH_2PO_4 and 12.54 g K_2HPO_4 in 90 ml deionized water. After the salts have dissolved, adjust the volume of the solution to 100 ml with deionized water and autoclave).

2X YT: Per liter:

To 900 ml of deionized water add:

Bacto tryptone	16 g
Bacto yeast extract	10 g
NaCl	5 g

Stir until dissolved. Adjust the pH to 7.0 with 5 N NaOH. Adjust the volume of the solution to 1 liter with deionized water and autoclave.

Glucose: Addition of 0.5% glucose to the medium is recommended to minimize expression prior to induction for IPTG-inducible expression systems susceptible to catabolite repression (5). The use of glucose may also increase the growth rate of the bacteria.

Important: Do not add glucose to the growth medium when using Overnight Express™ Autoinduction System 1.

Overnight Express Autoinduction System 1

Aeration: Efficient growth to saturation and utilization of carbon sources in OnEx™ Solution 1 requires vigorous agitation and proper aeration. Optimized culture volume:vessel dimension ratio is required in order to achieve proper aeration.

Temperature and length of incubation: It is important to grow the cultures to stationary phase when using the Overnight Express System 1. Using the cell culture guidelines above, stationary phase is usually reached as quickly as 8–10 hours, if the cultures are incubated at 37°C. When lower incubation temperatures are used, stationary phase may not be reached until 24 hours. Continued incubation for several hours after stationary phase appears to not be deleterious. Growth and induction at 25°C or 30°C may be optimal if you want to export the target using the signal sequence leaders present in a number of pET vectors or improve the yield of soluble protein.

Bacterial strains: Because lactose is used for induction, expression hosts should produce functional Lac permease (encoded by the *lacY* gene) and β-galactosidase (encoded by the *lacZ* gene) for consistent results in both complex and defined media. *lacY* mutant strains will not efficiently transport lactose for induction and *lacZ* mutants will not convert a portion of the transported lactose into the allolactose inducer. Elevated levels of target gene expression in *lacY*

and *lacZ* mutant strains may occur as cells approach stationary phase in some complex media. However, this induction will be variable depending upon medium composition, cell growth stage, and nutrient availability, all of which affect pH and the levels of cyclic AMP and acetate (6).

PopCulture® Reagent compatibility and optimization

Volume of PopCulture Reagent: There are no adverse effects to using higher ratios of PopCulture Reagent per ml of culture.

Bacterial strain: Protein extraction is efficient for both B (e.g., BL21) and K-12 (e.g., NovaBlue) strains. Other B and K-12 strains should be compatible; however, it is possible that strain-dependent differences could occur. It is important to consider the growth rate of the bacteria if performing the entire experiment in one day. BL21 and its derivatives have vigorous growth rates and are ideal for this extraction method.

Temperature of extraction: PopCulture extraction along with lysozyme and Benzonase Nuclease treatment can be performed at room temperature or at 4°C. However, incubation times may need to be increased because rLysozyme Solution and Benzonase Nuclease activities are decreased at lower temperatures.

pH of extraction: Acidic pH (< 5.0) can degrade components of PopCulture.

Protein assays: Because proteins generally retain their activities and conformation, protein-specific activity and immunoassays are likely to be compatible with PopCulture extraction. PopCulture treated extracts are compatible with the GST•Tag™ Assay, S•Tag™ Rapid Assay and FRETWorks™ S•Tag Assay Kits and BCA protein assays. Direct analysis of the extract by SDS-PAGE with Coomassie blue staining generally requires load the maximum well volume of crude extract combined with loading buffer containing a highly expressed target protein.

After purification with GST•Bind Resin, the elution fraction containing glutathione will not be compatible with BCA, Lowry, and GST•Tag protein assays. Note that the Bradford method and S•Tag Assays are compatible with glutathione. Proteins purified with Ni-NTA His•Bind Resin are compatible with BCA, GST•Tag, and S•Tag Assays.

Protease inhibitors: Protease inhibitors may be added with the PopCulture Reagent to the culture medium. Note that protease inhibitor cocktails that include EDTA are not compatible with Ni-NTA His•Bind Resin purification.

rLysozyme™ Solution

The addition of lysozyme increases the efficiency of cell lysis and protein extraction (4, 7, 8). rLysozyme Solution can be added separately or T7 lysozyme can be present in the cell prior to PopCulture Reagent treatment by incorporating pLysS or pLysE plasmid in the expression host strain with PopCulture Reagent. Inclusion of the pLysS or pLysE plasmid reduces the number of manipulations during protein extraction and purification. The pLysS and pLysE plasmids are based on the pACYC184 backbone and have the P15A replicon making them compatible with the pBR322 and pUC derived expression plasmids such as pET. pLysS and pLysE plasmids are maintained during culture in the presence of a final concentration of 34 µg/ml chloramphenicol.

rLysozyme Solution is effective using 40 U (1 µl rLysozyme Solution, diluted 1:750) per 1 ml initial culture volume. rLysoyzme Solution can be diluted using rLysozyme Dilution Buffer. Dilutions should be stored on ice or at 4°C until use and used as soon as possible. Do not store diluted rLysozyme Solution at –20°C because freezing may result in loss of activity.

Benzonase® Nuclease

Cell extracts may become viscous from nucleic acids released during cell lysis from a high density culture and/or when lysozyme is used. These nucleic acids can interfere with effective protein purification, especially with conventional purification resins. Benzonase Nuclease will degrade all forms of DNA and RNA (single stranded, double stranded, linear and circular) to 5'-monophosphate terminated oligonucleotides 2–5 bases in length (9, 10). Although Benzonase Nuclease requires Mg^{2+} for activation, it does not appear to require additional Mg^{2+} under the conditions described here for effective viscosity reduction and nucleic acid digestion. Benzonase® Nuclease treatment is not generally recommended for purification of proteins that

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must be nuclease-free. However, depending on the processing methods, Benzonase Nuclease may be removed during purification by anion exchange chromatography. Residual nuclease activity can be checked by incubation of the purified protein with RNA or DNA markers followed by gel analysis. For further details on Benzonase Nuclease see Technical Bulletin 261.

References

1. Studier, F. W. Personal communication.
2. Grabski, A., Mehler, M., and Drott, D. (2003) *in**Nova**tions* **17**, 3–6.
3. Tartoff, K. D. and Hobbs, C. A. (1987) *Focus* **9**, 12.
4. Grabski, A., Mehler, M., Drott, D., and Van Dinther, J. (2002) *in**Nova**tions* **14**, 2–5.
5. Novy, R. and Morris, B. (2001) *in**Nova**tions* **13**, 8–10.
6. Grossman, T. H., Kawasaki, E. S., Punreddy, S. R., and Osburne, M. S. (1998) *Gene* **209**, 95–103.
7. Grabski, A., Drott, D., Handley, M., Mehler, M., and Novy, R. (2001) *in**Nova**tions* **13**, 1–4.
8. Inouye, M., Arnheim, N., and Sternglanz, R. (1973) *J. Biol. Chem.* **248**, 7247.
9. Nestle, M. and Roberts, W. K. (1969) *J. Biol. Chem.* **244**, 5213–5218.
10. Janning, P., Schrader, W., and Linscheid, M. (1994) *Rapid Commun. Mass Spectrom.* **8**, 1035–1040.