PopCulture™ Reagent

About the Kit

PopCulture Reagent	15 ml	71092-3
	75 ml	71092-4
	250 ml	71092-5

PopCulture Reagent* is a buffered mixture of concentrated detergents formulated to extract proteins from *E. coli* cells directly in their culture medium. Using this method, cell culture, protein extraction and purification performed in the original culture tube or multiwell plate. PopCulture perforates the *E. coli* cell wall without denaturing soluble protein and protects protein from the pH extremes produced in high density culture media. Recombinant proteins can be assayed directly or purified by adding an affinity matrix, washing the matrix:target protein complex to remove culture medium and cellular contaminants and eluting the purified protein from the matrix. Purification of fusion proteins from total culture extracts has been demonstrated using both IMAC and GST affinity chromatography methods (Grabski et al., 2001).

To further enhance the PopCulture purification procedure, lysozyme and/or Benzonase® Nuclease may be added. Lysozyme cleaves a peptidoglycan bond in the *E. coli* cell wall, enhancing cell lysis and increasing the yield of protein (Inouye et al., 1973; Grabski et al., 2001). Proteins may be expressed in a host encoding T7 lysozyme (pLysS host) or exogenous lysozyme may be added after the PopCulture Reagent. Benzonase Nuclease may also be added to degrade endogenous nucleic acids that may interfere with purification due to high viscosity.

The PopCulture protein purification procedure is ideally suited to high throughput (HT) robotic processing of samples for proteomics research or any application that would benefit from the increased speed and convenience. The magnetic agarose capture resins (e.g. GST•Bind™ Magnetic Agarose Beads, His•Bind® Magnetic Agarose Beads) are optimal for HT applications since the entire procedure can be performed in a single tube without the need for columns or centrifugation.

Storage

Store the PopCulture Reagent at room temperature. Slight turbidity may result if PopCulture is stored at 4°C. If this occurs, equilibrate to room temperature (23°C) and mix by inversion prior to use.

* patent pending

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Protocol

1. Culture *E. coli* transformed with an expression plasmid under standard conditions for target protein expression. See *"Growth and Induction"* below for more information.
2. Add 0.1 culture volume of PopCulture Reagent.
3. Mix and incubate for 10 min at room temperature.
4. Optional: Add lysozyme and/or Benzonase® Nuclease, mix and incubate 15 min at room temperature.
   Benzonase Nuclease: Use 1 µl (25 U) per 1 ml of the original culture volume.
   Lysozyme: Use 2.5–5 µg of chicken egg lysozyme per 1 ml of original culture volume. Use a freshly prepared stock (1 mg/ml) of chicken egg lysozyme in water and keep at 4°C or on ice until use. Additional lysozyme is not necessary if the host strain contains the pLysS plasmid.

Note: Analysis of the target protein expression by SDS-PAGE with Coommassie blue staining is not recommended because the target protein is at a low concentration in the culture medium.

5. After the PopCulture incubation is complete, this prepared extract can be combined with an equilibrated purification resin. Follow the standard protein purification procedures according to the type of resin used. For protocols incorporating the His•Bind® or GST•Bind™ resins, see Novagen's Technical Bulletins 054 and 236 respectively.

Growth and Induction

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. In general, BL21 and its derivatives have a vigorous growth rate and are ideal for this extraction method. The use of lysozyme expressing hosts like BL21(DE3)pLysS increase the protein extraction efficiency without the addition of exogenous lysozyme.

Medium: Luria-Bertani (LB) Terrific Broth (TB, Tartof and Hobbs, 1987) and 2X YT media have all been used successfully with PopCulture.

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	2 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 of sterile 0.17 M KH_2PO_4, 0.72 M K_2HPO_4 solution (dissolve 2.31 g of KH_2PO_4 and 12.54 g of K_2HPO_4 in 90 ml of 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.

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Cell Culture: The entire procedure from culturing through purification can be done in one day. Therefore it is ideal for small culture volumes (≤ 50 ml) or multiwell plate cultures. For example, take a single colony or several colonies of the expression host from a freshly streaked plate (with appropriate antibiotics) and inoculate 3 ml of pre-warmed medium. After the OD_{600} reaches 0.8–1, inoculate 50 ml medium with the entire 3 ml or let the culture grow until the OD_{600} reaches 0.8–1.5 and then induce. Use pre-warmed medium to avoid a lag phase in bacterial growth. Note that bacterial strains with a slower growth rate may be difficult to use in a one day procedure. Alternatively, grow a culture overnight at a reduced temperature (24–26°C) and induce the following day after raising the temperature to 37°C and growing to an appropriate OD_{600} for induction.

Inclusion of 0.5% glucose is recommended to minimize expression prior to induction for those expression systems susceptible to catabolite repression (Novy and Morris, 2001). The inclusion of glucose may also increase the growth rate of the bacteria. Review the pET System Manual (Technical Bulletin 055) or *Current Protocols in Protein Science* for additional cell culture guidelines for protein expression.

Additional Guidelines for PopCulture Reagent optimization and compatibility

Lysozyme: Experiments with PopCulture show that the addition of lysozyme increases the efficiency of cell lysis and protein extraction (Grabski et al., 2001). Chicken egg lysozyme can be added separately or T7 lysozyme can be present in the cell prior to PopCulture treatment by incorporating the pLysS plasmid in the expression host strain.

Inclusion of the pLysS plasmid reduces the number of manipulations during protein extraction and purification. The pLysS plasmid is based on the pACYC184 backbone and has the p15A origin of replication, making it compatible with the pBR322 and pUC derived expression plasmids such as pET and pTriEx™. The pLysS plasmid is maintained during culturing with a final concentration of 34 µg/ml chloramphenicol.

Chicken egg lysozyme (Cat No. 4403) is effective in the range of 2.5–5 µg per 1 ml of initial culture volume. Prepare a fresh 1 mg/ml stock of chicken egg lysozyme with water for each experiment. Store the lysozyme on ice or at 4°C until use.

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. It may not be as critical to reduce the viscosity with magnetic purification methods. The addition of Benzonase Nuclease (Cat. No. 70746) 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 (Nestle and Roberts, 1969, Janning et al, 1994). Although Benzonase Nuclease requires Mg^{2+} for activation, it does not appear to require additional Mg^{2+} under the conditions described here. The activity is sufficient for effective viscosity reduction and nucleic acid digestion. Benzonase Nuclease treatment is not generally recommended for purification of proteins that 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.

Volume of PopCulture Reagent: There are no adverse effects to using larger volumes of PopCulture Reagent. If the cells need to be concentrated prior to extraction, the volume of PopCulture can be adjusted accordingly.

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

pH of Extraction: Acidic pH can degrade components of PopCulture.

Reducing agents: PopCulture is compatible with reducing agents such as 2-mercaptoethanol and DTT. Note that reducing agents may activate proteases and will interfere with protein binding to some purification resins (e.g. His•Bind Resin but not Ni-NTA His•Bind Resin).

EDTA: Although EDTA is compatible with PopCulture, it is not compatible with the His•Bind Resin and, although not recommended, may be compatible up to 1 mM with Ni-NTA His•Bind Resin. Benzonase Nuclease is inhibited by EDTA at concentrations > 1 mM due to chelation of essential Mg^{2+} ions.

Protein assays: PopCulture is compatible with the GST•Tag™ Assay, S•Tag™ Rapid Assay and FRETWorks™ S•Tag Assay Kits.

Protease inhibitors: Protease inhibitors may be added with the PopCulture Reagent to the culture medium. Serine protease inhibitors should be avoided if the target protein is to be treated with the serine proteases thrombin, Factor Xa or enterokinase. Active inhibitor may be carried through the purification process and affect cleavage reactions. Note that protease inhibitor cocktails which include EDTA would not be compatible with His•Bind Resin.

Purification resins: The PopCulture Reagent contains Tris buffer and is compatible with both Tris (His•Bind) and phosphate-buffer (GST•Bind and Ni-NTA) purification systems at a neutral pH. Although PopCulture successfully buffers the extract, there may be experiments when the pH of the extract is below pH 7.0. In this case the extract must be adjusted to a pH of 7.5 by the addition of 1 M Tris-HCl pH 8.0 prior to loading on any of the His•Bind supports with IDA chemistry (His•Bind Resin, His•Bind Columns, His•Bind Fractogel, His•Bind Quick Resins and His•Bind Magnetic Agarose Beads). PopCulture is expected to be compatible with many other affinity purification resins. The effect of high salt buffer compositions or acidic pH ranges for chromatography on the PopCulture method should be carefully evaluated. PopCulture will precipitate at or above 1 M NaCl. Attention should be paid to any observed precipitation of the PopCulture reagent or target protein, and compatibility of the PopCulture reagent with buffers used for equilibration of chromatography columns. For more information using the His•Bind and GST•Bind Resins with PopCulture Reagent, see Technical Bulletins 054 (His•Bind) and 235 (GST•Bind).

References

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