LYTAG TWO-PHASE

LYTAG TWO-PHASE PURIFICATION SYSTEM

LYTAG Two-Phase is a protein purification system based on the use of two aqueous components. The method relies on the affinity of the protein tag LYTAG for one of the two-phase components, allowing recombinant protein separation and purification from cellular extracts or culture media. In the procedure, the LYTAG-fused protein is retained in one of the aqueous phases while most of the undesired proteins can be removed by simply discarding the opposite phase. After replenishing the system with fresh phase, the protein of interest can be easily recovered in it, with high purity, by reversing its localization with the addition of choline, the specific LYTAG ligand.

This system is particularly well suited for industries and laboratory specialized in protein separation and purification, as it is simple, cost efficient, time saving and highly versatile for scaling up protein purification process, representing a convenient dternative to solid resins.

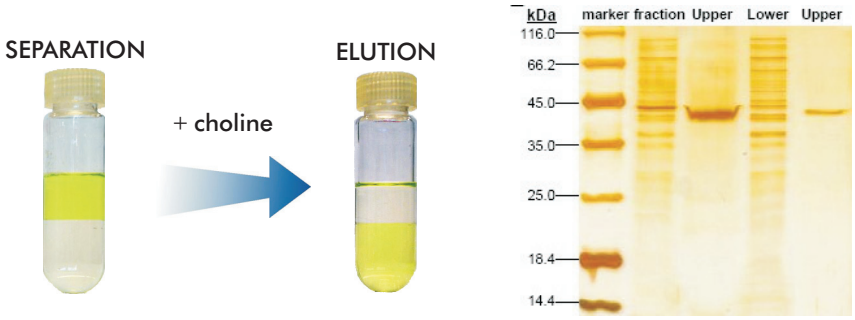

Figure 1. Purification of a GFP-LYTAG fusion protein expressed with the CASCADE™ system in the *E. Coli* host strain REG1. A 10 ml crude cell lysate was prepared from a 250 ml culture, and mixed with Top Lower Solutions. After separation of the two phases, addition of choline led to migration of the GFP-LYTAG fusion protein, from the upper to the lower phase.

Application:

– Rapid and scalable purification of recombinant LYTAG fusion proteins.

Advantages:

– **Quick method.** Separation can be completed within few minutes, minimizing the effects of proteases. It is specially convenient when large volumens (>10 ml) of cellular extracts or culture media need to be processed (usually requiring prolonged flow times when using chromatography columns).

– **Easy** and **inexpensive** protein purification process.

– No special equipment requirements.

– It offers, like every aqueous two-phase systems, **mild conditions** in the separation of labile proteins. Purification can be easy and safely performed at low temperatures, requiring only a refrigerated centrifuge and an ice water bath.

– **Scalable** method.

– **High purification efficiency** (>95% purity), comparable to the use of solid matrixes.

– Optimum performance in downstream fermentation processes.

– Good alternative to conventional solid resins.

TECHNOLOGY OFFER

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Cell extract volumes	Resin	LYTAG 2-Phase
10 ml	30-60 min	15 - 30 min
50 ml	1,5 - 2 h	15 - 30 min
100 ml	3 - 4 h x4	15 - 30 min
200 ml	6 - 7 h x7	15 - 30 min

Resin

Flow rate: 1ml/min

LYTAG 2-Phase

Centrifugation: 5 min

GENERAL PROCEDURES

1. Use a transparent centrifuge tube to mix 1 volume of crude lysate or culture medium with 1 volume of Top Solution and 0.5 volumes of Lower Solution. Mix by inverting the tube to obtain a homogeneous suspension.

2. Centrifuge at 10000 xg for 5 min, 4 °C.

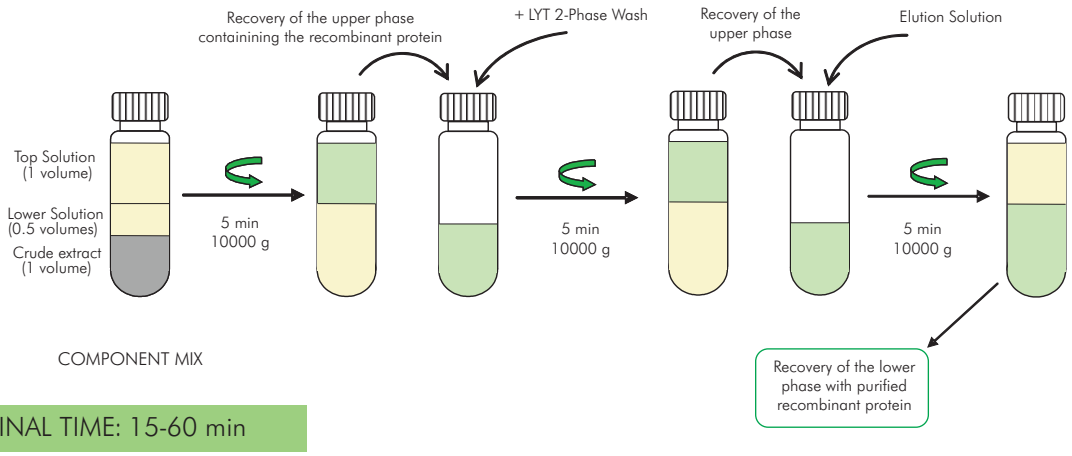
3. Using a pipette, transfer the upper phase to a clean centrifuge tube. Discard the lower phase.

4. Wash[a]. Prepare 1x LYT 2-Phase Wash Solution by diluting the provided 3x stock in distilled water, and add a volume equivalent to that os the discarded phase, to the upper phase. Mix by inverting the tube and centrifuge at 10000 xg for 5 min, 4 °C.

5. Repeat step 3.

6. Elution to lower phase. Prepare Elution Solution byluting the provided 3M Choline Chloride solution in LYT-2 Phase Wash Solution (1 volume LYT-2 Phase Wash(3x) : 0.15 volumes 3M Choline Chloride: 1.85 volumes distilled water), and add a volume equivalent to that of the discarded phase, to the upper phase. Mix thoroughly by inverting the tube and centrifuge inmmediately at 10000 xg for 5 min, 4 °C. Transfer the upper phase[b] to a different tube and check for the presence of the recombinant protein in the lower phase.

([a]) Wash steps 3 and 4 may be repeated if a higher degree of purity is required.
([b])Recovery of recombinant protein from the upper phase can be increased by repeating the elution process in step 6.



FINAL TIME: 15-60 min

Figure 2. General procedure. Processing time is independent of initial lysate volume.

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