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 alternative to solid resins.

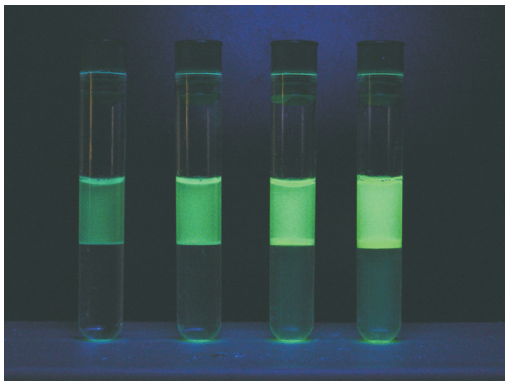

Figure 1. Protein separation of different concentrations of a GFP-LYTAG fusion protein by affinity two liquid phase partitioning.

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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LYTAG TWO-PHASE PURIFICATION SYSTEM

Cell extract volumes	Resin	LYTAG Two-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 Two-Phase

Centrifugation: 5 min

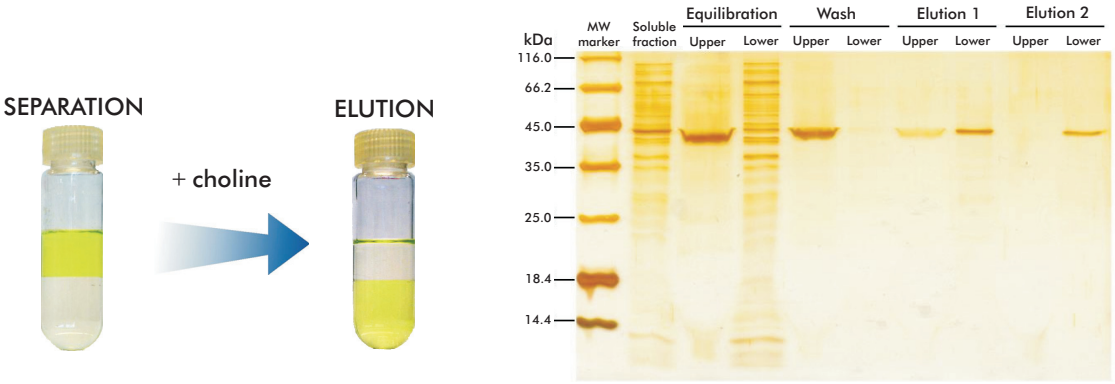

Figure 2. Purification of a GFP-LYTAG fusion protein expressed with the CASCADE™ Expression System. Crude cell lysate (10 ml) was prepared from a 250 ml culture, and mixed with Top/Lower Solutions. After separation of the two phases, addition of choline led to migration of the GFP-LYTAG fusion protein, from the upper to the lower phase.

KIT COMPONENTS

Reagents provided	Size				Storage
	For 20 ml cellular extract		For 80 ml cellular extract		
	QUANTITY	CAT.N°	QUANTITY	CAT.N°	
Top Solution	20 ml	RS-4751	80 ml	RS-4740	RT
Lower Solution	10 ml	RS-4752	40 ml	RS-4741	RT
LYTAG Two-Phase Wash Buffer (2x)	65 ml	RS-4753	240 ml	RS-4754	RT
3M Choline Chloride	10 ml	RS-4755	25 ml	RS-4756	4°C
1M Salicylate (inducer)	25 ml	RS-3247	25 ml	RS-3247	4°C
pALEX2a, b, c	8 µg (each)	EV-4658	8 µg (each)	EV-4658	-20°C
pALEX2-Ca-GFP (control)	8 µg	EV-4757	8 µg	EV-4757	-20°C
E.coli REG12	Stab	BS-3458	Stab	BS-3458	4°C

References	Product	Price
KT-4697	LYTAG Two-Phase Purification System 20 ml	851 €
KT-4739	LYTAG Two-Phase Purification System 80 ml	990 €

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