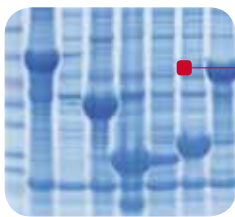
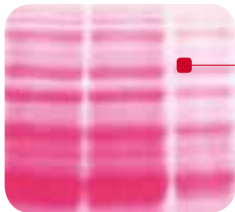


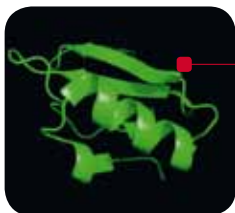
Protein



Expression



Extraction



Refolding

We offer you

- Protein expression: *E.coli*, Yeast and Mammalian expression systems
- Protein extraction: Protein extraction of integral membrane proteins and from fungi and bacteria
- Protein refolding: Refolding of cytosolic and membrane proteins

Protein expression

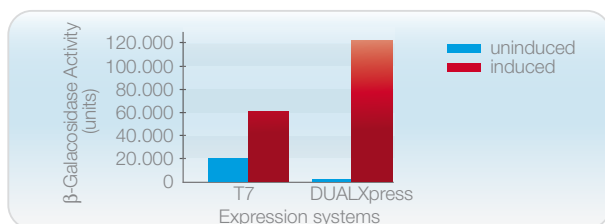
• *E. coli* expression systems

DUALXpress protein expression starter kit

High speed, high yield protein expression

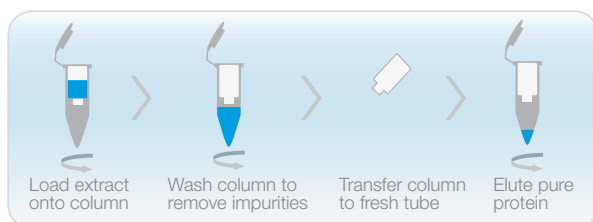
- Ultra-tight regulation and high-level expression of recombinant proteins
- Increased expression of proteins in the soluble fraction
- One-step purification from crude lysates yields > 95 % pure protein
- DualTRAP resin is highly selective, resulting in fewer background contaminants
- Elution buffer does not interfere with downstream protein quantification
- LYTAG is easily removed by Enterokinase cleavage
- N- and C-terminal LYTAG vectors available

Technology — Comparison of DUALXpress and T7 expression systems



The DUALXpress system achieves tight regulation and exceptionally high induction of expression using the regulatory circuit of the salicylate-responsive activators of *Pseudomonas putida*. The result is a very high signal to noise ratio.

Easy purification using a table top centrifuge



• Ordering Information

P01008 | DUALXpress protein expression starter kit
P08001 | QuickLyse pEAS-1a expression vector
P01006 | KickStart protein expression kit
P03301 | p427-TEF
P03302 | p417-CYC
P03303 | pTEF-MF
P03304 | pGAL-MF

The QuickLyse system

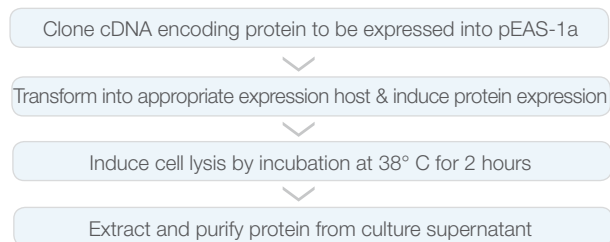
Rapid autolysis of bacterial strains for recombinant protein purification



- Suitable for high-throughput protein expression
- Recombinant proteins are purified directly from the culture supernatant
- No need for lysozyme treatment or sonication
- Gentle extraction conditions ensure integrity and activity of the recombinant protein

Expression procedure

The system is compatible with common *E. coli* expression strains, such as BL21 or XL1-Blue.



References

Kloos *et al.* (1994) *J. Bacteriol.* 176:7352-7361
Xu *et al.* (2006) *Biotechniques* 41:319-322

Interested?

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P04003 | DSY-5 yeast expression strain
P04007 | DSY-6 yeast expression strain
P03401 | pHA-MEX
P03402 | pMEX-HA
P01013 | TOTAL protein extraction kit
P07002 | DUALXtract buffer set
P07201 | DUALrefold soluble protein refolding kit
P07501 | DUALrefold membrane protein refolding kit

■ Yeast expression systems

KickStart protein expression kit

High level, inducible protein expression in yeast

- High-level, inducible expression in *S. cerevisiae*
- No addition of reagents or change of medium needed for induction
- Tight regulation of induction allows expression of toxic proteins
- Protease deficient expression strain for higher protein yields
- Separation of growth and production phase

Yeast expression vectors

p427-TEF

High copy yeast expression vector carrying the aminoglycoside phosphotransferase (KanMX) gene for selection in yeast using G418. Inserts are expressed from the strong TEF promoter.

p417-CYC

Low copy yeast expression vector carrying the aminoglycoside phosphotransferase (KanMX) gene for selection in yeast using G418. Inserts are expressed from the weak CYC1 promoter.

pTEF-MF

Yeast expression vector for secreted proteins. A strong TEF1 promoter drives constitutive expression of a cDNA fused to the pre-pro leader sequence of mating factor alpha to ensure secretion of the protein product into the medium.

pGAL-MF

Yeast expression vector for secreted proteins. A regulatable GAL-L promoter drives inducible expression of a cDNA fused to the pre-pro leader sequence of mating factor alpha to ensure secretion of the protein product into the medium.

■ Mammalian expression systems

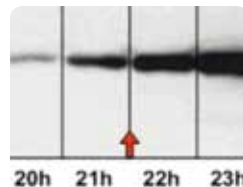
Mammalian expression vectors

Mammalian expression vectors for stable or transient expression of cDNAs in mammalian cells:

- Strong CMV promoter for high-level, constitutive expression
- HA epitope tag for convenient detection of expression products
- Sfi I sites for convenient, directional cloning of full-length cDNAs

Technology

Auto-inducible ADH2 promoter:



The ADH2 promoter is repressed by glucose. When the cells reach the early stationary phase, glucose is depleted from the medium (red arrow) and the ADH2 promoter is induced, producing high amounts of protein.

KanMX cassette: KickStart vectors feature a KanMX cassette, which allows selection with G418 in rich medium. The use of rich medium such as YPD significantly enhances cell densities and protein yield.

Yeast expression strains

DSY-5 yeast expression strain

General purpose strain for expression of recombinant proteins in yeast. Deletion of *prb1* encoding the vacuolar proteinase B (*yscB*) and *pep4* encoding the vacuolar aspartyl protease (proteinase A) ensures good yield and little non-specific degradation when purifying proteins from yeast. Can be used in combination with our general purpose yeast expression vectors.

MAT α *leu2 trp1 ura3-52 his3::GAL1-GAL4 pep4 prb1-22*

DSY-6 yeast expression strain

DSY-6 has been designed for expression of recombinant proteins in yeast. Good growth characteristics ensure high cell densities both in synthetic defined (SD) and rich (YPD) medium. Deletion of *prb1* encoding the vacuolar proteinase B (*yscB*), *pep4* encoding the vacuolar aspartyl protease (proteinase A) and *prc1* encoding the vacuolar carboxypeptidase Y (proteinase C) ensures minimal protein degradation during cell lysis and protein extraction.

MAT α *leu2 trp1delta63 ura3-52 prb1-22 pep4-3 prc1-407*

pHA-MEX

Mammalian expression vector featuring a strong CMV promoter, an N-terminal HA epitope tag for detection of expressed proteins and a NeoR cassette for stable integration of the expression vector into the genome of the host cell.

pMEX-HA

Mammalian expression vector featuring a strong CMV promoter, a C-terminal HA epitope tag for detection of expressed proteins and a NeoR cassette for stable integration of the expression vector into the genome of the host cell.

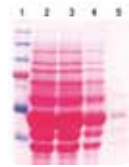
Protein extraction

Protein extraction from fungi and bacteria

TOTAL protein extraction kit

- Especially formulated for protein extraction from *E.coli* and yeast
- Complex detergent mix guarantees improved protein solubilization
- Enhanced extraction of strongly hydrophobic proteins such as membrane proteins
- Sufficient for 60 sample preparations

Comparison of extraction efficiencies



Lane 1: marker, Lanes 2 and 3: two independent extractions using the TOTAL protein extraction kit, Lanes 4 and 5: extractions using kits from competitors

Protein extraction of integral membrane proteins

DUALXtract buffer set

- Highly efficient, quantitative extraction of integral membrane proteins
- 12 different extraction conditions
- Non-denaturing detergents and synthetic lipid analogues provide gentle extraction and stabilization conditions

Step 1: Use the DUALXtract buffer set to test 12 different extraction buffers on your membrane protein of interest

Step 2: Once you have identified the optimal extraction buffer, use this buffer to perform a large scale extraction

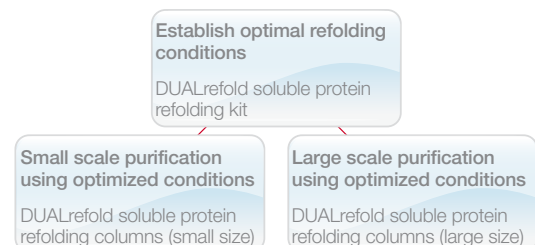
Step 3: Continue with downstream work using the extracted membrane protein

Protein Refolding

Refolding of cytosolic proteins

DUALrefold soluble protein refolding kit

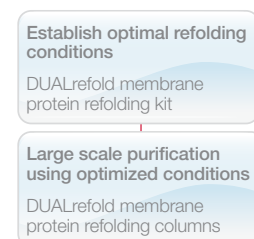
- Highly efficient refolding of denatured cytosolic proteins
- Test 10 different refolding conditions using the DUALrefold column set
- Once the optimal refolding conditions have been identified, large scale refolding can be performed



Refolding of membrane proteins

DUALrefold membrane protein refolding kit

- Highly efficient refolding of denatured membrane proteins
- Test 20 different refolding conditions using the DUALrefold column set
- Once the optimal refolding conditions have been identified, large scale refolding can be performed



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