



用户手册 第一版 2009 年 12 月

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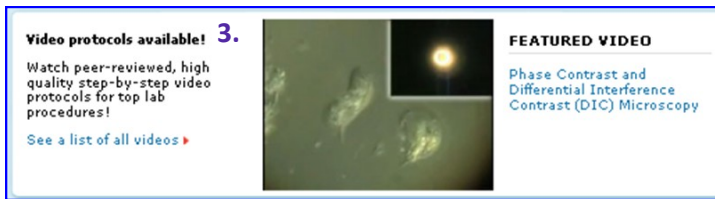
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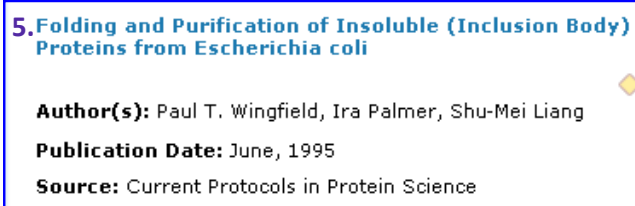
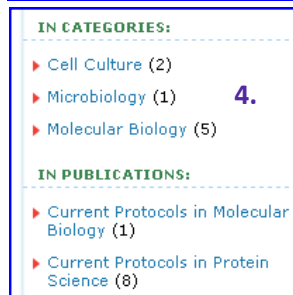
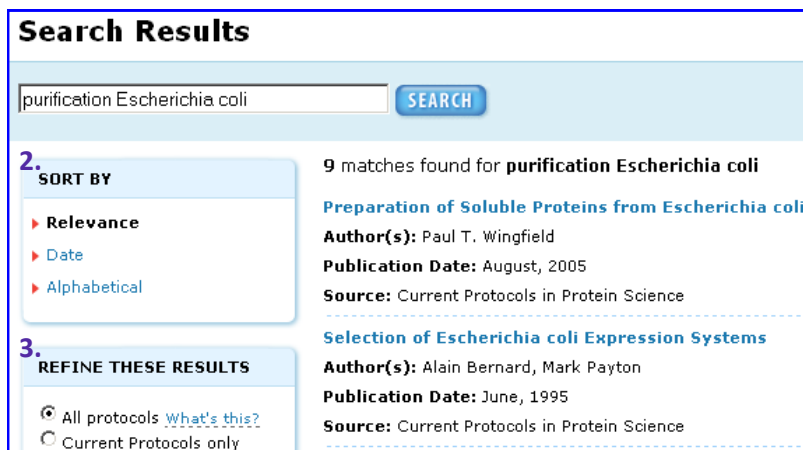
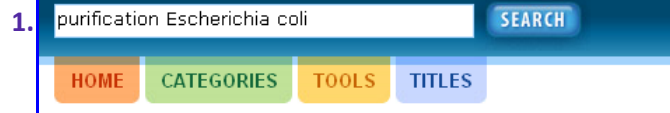
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Folding and Purification of Insoluble (Inclusion Body) Proteins from Escherichia coli

PEER REVIEWED

Paul T. Wingfield¹, Ira Palmer¹, Shu-Mei Liang²

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Publication Name: Current Protocols in Protein Science
Unit Number: UNIT 6.5
DOI: 10.1002/0471140864.ps0605s00
Print Publication Date: June, 1995
Online Posting Date: May, 2001

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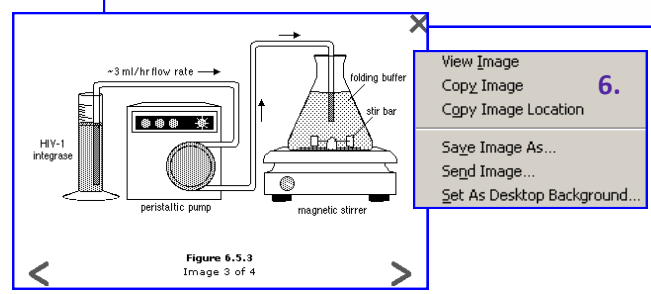
AUTHOR NOTES

ABSTRACT

Heterologous expression of recombinant proteins in E. coli often results in the formation of insoluble and inactive protein aggregates, commonly referred to as inclusion bodies. To obtain the native (i.e., correctly folded) and hence active form of the protein from such aggregates, four steps are usually followed: (1) the cells are lysed and the aggregates, (2) the cell wall and outer membrane components of the aggregates are removed, (3) the aggregates are solubilized (or extracted) with strong protein denaturants, and (4) the solubilized, denatured proteins are folded with concomitant oxidation of reduced cysteine residues into the correct disulfide bonds to obtain the native protein. This unit features three different approaches to the final step of protein folding and purification. In the first, guanidineHCl is used as the denaturant, after which the solubilized protein is folded (before purification) in an "oxido-shuffling" buffer system to increase the rate of protein oxidation. In the second, acetic acid is used to solubilize the protein which is then refolded in a buffer containing oxidizing agents and chaperones.

NOTE: All steps are carried at 4°C unless otherwise stated.
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Figure 6.5.3
Setup for folding of HIV-1 integrase by dilution into buffer.
5. View Image



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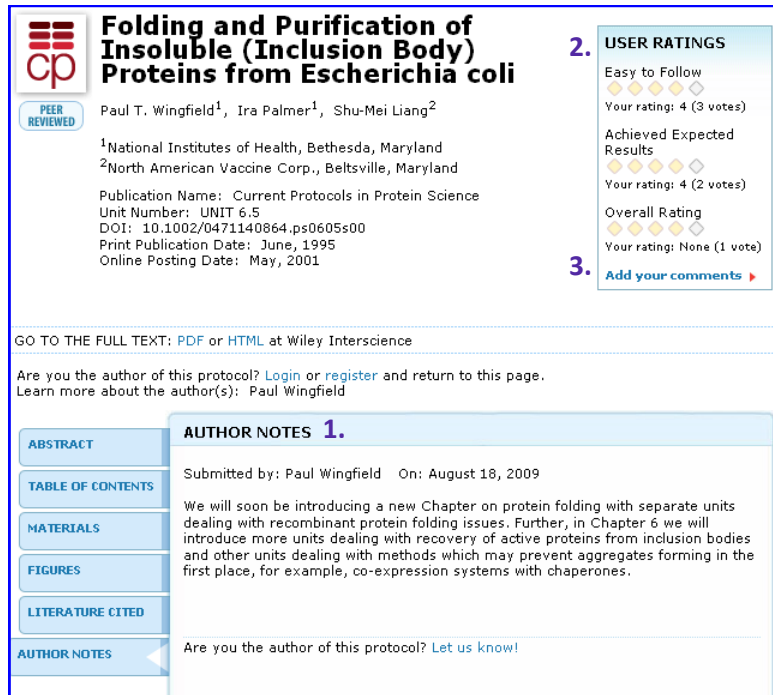
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Folding and Purification of Insoluble (Inclusion Body) Proteins from Escherichia coli

Paul T. Wingfield¹, Ira Palmer¹, Shu-Mei Liang²

¹National Institutes of Health, Bethesda, Maryland
²North American Vaccine Corp., Beltsville, Maryland

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AUTHOR NOTES 1.

Submitted by: Paul Wingfield On: August 18, 2009

We will soon be introducing a new Chapter on protein folding with separate units dealing with recombinant protein folding issues. Further, in Chapter 6 we will introduce more units dealing with recovery of active proteins from inclusion bodies and other units dealing with methods which may prevent aggregates forming in the first place, for example, co-expression systems with chaperones.

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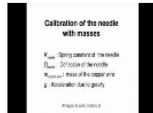
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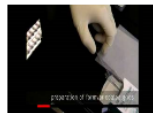
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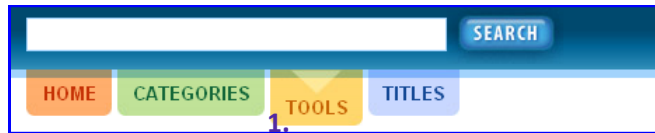
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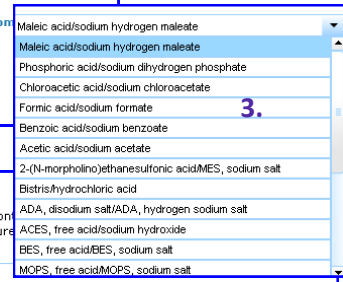
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Buffer Calculator

Provides recipes for the preparation of buffers over a concentration range of 0.001 to 1000 mol/l. It enables scaling for volume and correction for temperature. It lists the most commonly used buffer systems in order of ascending pKa's.

TO PREPARE Buffer: Maleic acid/sodium hydrogen maleate **3.** with pKa: 2.00

Concentration (mol/l): 0.005 2 0.005 0.005 2

Volume (ml): 100 2000 100 100 100

pH: 1 3 1 1

Temperature of usage (C°): 0 60 0 60

4.

FOLLOW THE RECIPE **5.** 0.0004645

Ingredient	Stock concentration (mol/l)	Volume (ml)
Maleic acid	0.005 <input type="text"/> 5 <input type="text"/> 1 <input type="text"/> 1	0.4536
Sodium hydrogen maleate	0.005 <input type="text"/> 5 <input type="text"/> 1 <input type="text"/> 1	0.04645

Add water up to: 100 ml

Check pH and correct it if necessary

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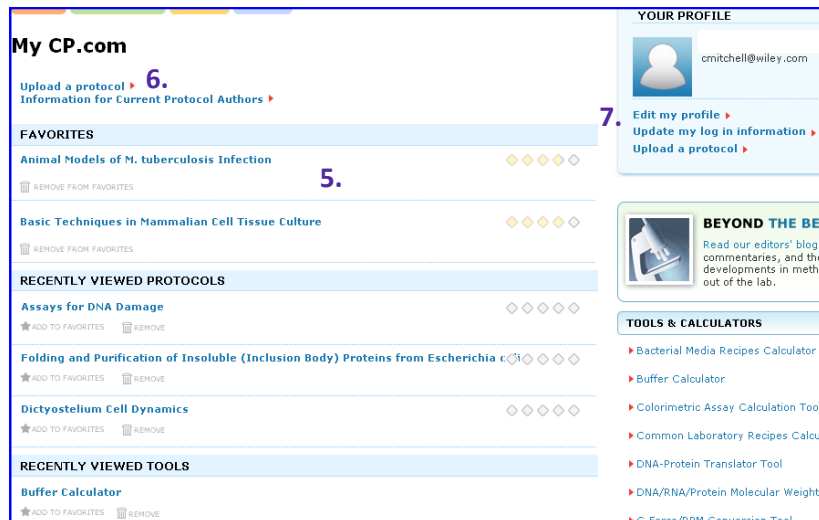
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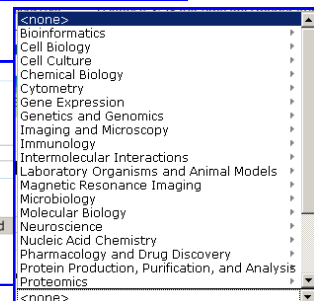
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PROTOCOL INFO *

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Select the protocol's suppliers or authors from the list. If not listed, use the Add button to create a new listing.

SUPPLIER/AUTHOR DETAILS

Supplier(s):

- None -

5.

Add a New Supplier

Author(s):

- None -

Add a New Author



6.

PROTOCOL SUBMISSION STATUS

- Draft (viewable by you only)
- Submitted for CP Editorial Approval (publicly viewable after approval)

Save & Preview **7.**

注释:

