



用户手册 第二版 2011 年 1 月

## 目录

## Page

<a href="#">关于实验室指南</a>	2
<a href="#">currentprotocols.com 网站功能</a>	3
<a href="#">主页</a>	4
<a href="#">注册和登录</a>	5
<a href="#">浏览</a>	6
<a href="#">搜索</a>	7
<a href="#">内容概览</a>	8
<a href="#">内容概览 &gt; 更多</a>	9
<a href="#">视频指南使用手册</a>	10
<a href="#">工具和计算器</a>	11
<a href="#">MyCP.com</a>	12
<a href="#">上传指南手册</a>	13

注释:

关于实验室指南.....

实验室指南是研究生物化学、分子生物学和/或生物医学问题的实验或“方法”，既严谨又详尽：

- 通过提供可靠的、经过检验的程序帮助您加快研究速度
- 它以标准格式编排，便于实验室环境中使用
- 分步详述的程序
- 收纳了所需的材料和设备
- 附有图示和表格
- “注释”或“提示”为疑难问题提供了建议



注释：

实验室指南产品网站的功能 [currentprotocols.com](http://currentprotocols.com).....

- 搜索和浏览所有指南
- 工具和计算器
- 视频指南手册
- 新指南提示服务和 RSS 订阅
- 网站上的所有内容均提供公开访问
- 可在 [Wiley InterScience](http://Wiley InterScience) 上找到全文（通过订阅）
- 可加入实验仪器及实验相关药品材料供应商和使用者制作的实验指南
- 指南中附有评价和注释
- “Ask the Experts (咨询专家)” — 免费的在线指南建议
- 疑难解答论坛
- 阅读编辑博客 — 获取新闻、注释和最新的方法发展动态
- 通过 [My CP.com](http://My CP.com) 定制属于自己的浏览内容
  - 上传自己的指南，与实验室指南社区实现共享
  - 编辑自己的最爱收藏

#### TOOLS & CALCULATORS

- ▶ [Bacterial Media Recipes Calculator](#)
- ▶ [Buffer Calculator](#)
- ▶ [Colorimetric Assay Calculation Tool](#)
- ▶ [Common Laboratory Recipes Calculator](#)
- ▶ [DNA-Protein Translator Tool](#)
- ▶ [DNA/RNA/Protein Molecular Weight Calculator](#)
- ▶ [G-Force/RPM Conversion Tool](#)
- ▶ [Hemocytometer Calculation Tool](#)
- ▶ [ImageJ Java Applet](#)
- ▶ [NEBcutter v. 2.0](#)
- ▶ [Polyacrylamide Gel Recipes Calculator](#)
- ▶ [Primer3Plus](#)
- ▶ [Radioactive Decay Calculator for Isotopes Commonly Used in Biomedical Research](#)
- ▶ [Solution Concentration Calculator](#)
- ▶ [Spectrophotometric Measurement of Nucleic Acids Calculator](#)
- ▶ [Units of Measurement Conversion Tool](#)

#### DISCUSSION BOARDS

- ▶ [General Discussion](#)
- ▶ [Ask the Experts: Molecular and Cell Biology](#)
- ▶ [Ask the Experts: Protein Science](#)

#### Video protocols available!

Watch peer-reviewed, high quality step-by-step video protocols for top lab procedures!

[See a list of all videos ▶](#)



#### FEATURED VIDEO

[Phase Contrast and Differential Interference Contrast \(DIC\) Microscopy](#)

注释:

主页

进入主页后您可以搜索和浏览，访问讨论版块、工具、计算器和编辑博客，浏览视频指南，建立新的指南提示服务或 RSS 订阅

1...直接通过目录从主页进行浏览，或点击任何页面的“CATEGORIES”按钮即可浏览。

2...您可以在主页上或任意页面上运用简单搜索文本框进行搜索

3...浏览专题视频指南或链接查看完整列表。

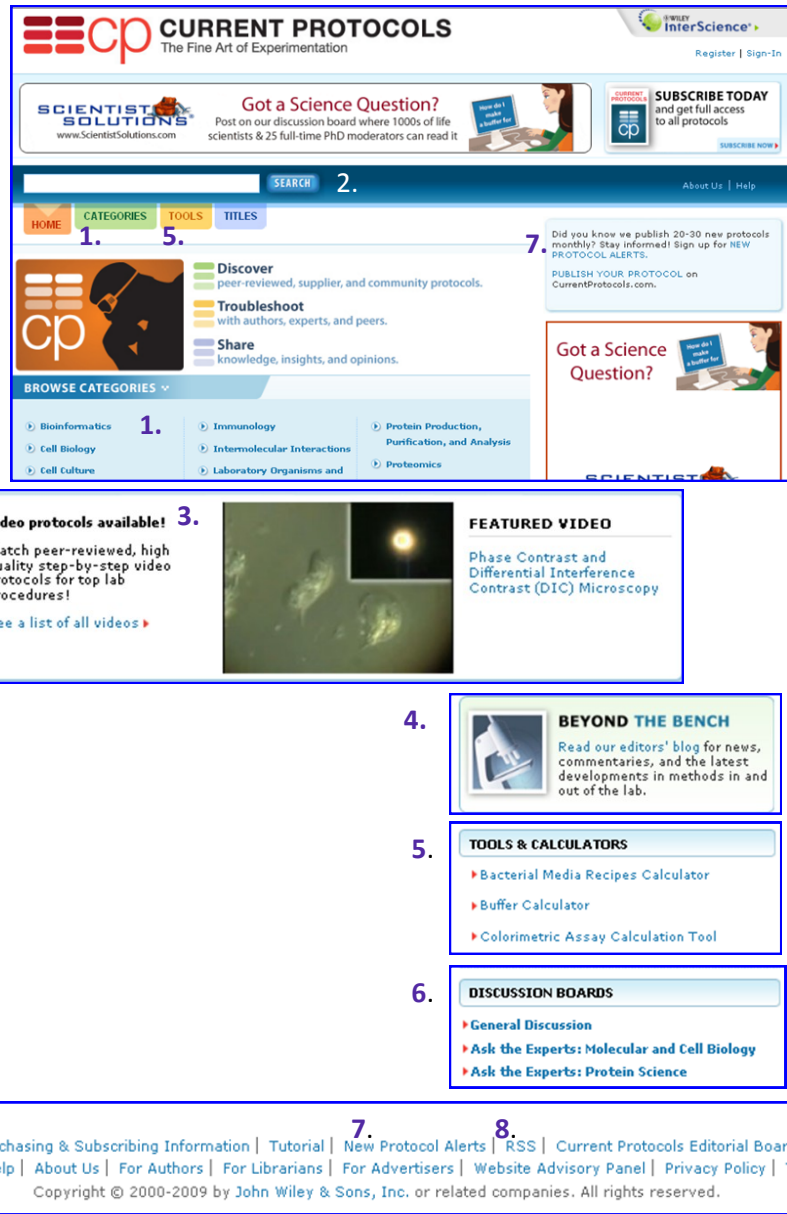
4...在所有页面右边都可链接到编辑博客-BEYOND THE BENCH。

5...您可以通过“TOOLS”按钮，和所有页面右边链接到TOOLS & CALCULATORS。

6...您可以从所有页面右边链接进入“DISCUSSION BOARDS”。

7...在屏幕右边或滚动至页面底部设置邮件提示服务。

8...您还可以设置 RSS 订阅。



注释：

主页 > 注册和登录

在 [currentprotocols.com](http://currentprotocols.com) 上注册后您就可以添加和整理自己的最爱收藏，并上传自己的指南与实验室指南社区实现共享。

1... 点击主页上的注册链接。

2... 在注册页面上输入您的邮件地址和密码。

3... 输入您的详细个人信息。

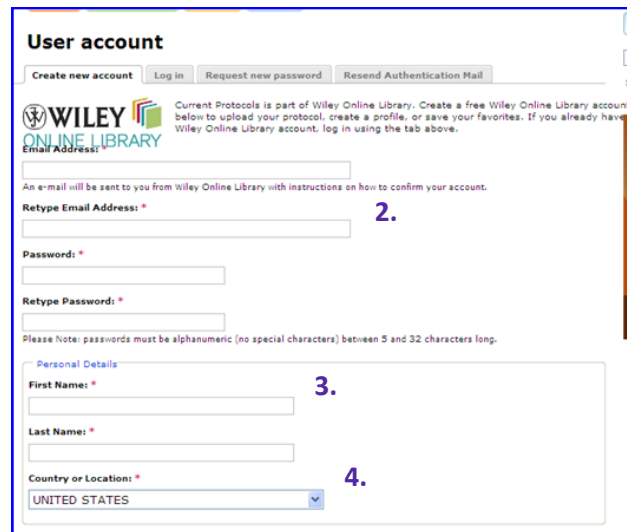
4... 输入您所在的国家或地区。

5... 完成安全检查。

6... 核对您的详细信息并点击“Register”。

提交后，您将收到一封邮件，需要您在 24 小时之内核对账户信息。

7... 核对账户后您就可以登录了。



**User account**

Create new account | Log in | Request new password | Resend Authentication Mail

**WILEY ONLINE LIBRARY** Current Protocols is part of Wiley Online Library. Create a free Wiley Online Library account below to upload your protocol, create a profile, or save your favorites. If you already have a Wiley Online Library account, log in using the tab above.

Email Address: \*

An e-mail will be sent to you from Wiley Online Library with instructions on how to confirm your account.

Retype Email Address: \* 2.

Password: \*

Retype Password: \*

Please Note: passwords must be alphanumeric (no special characters) between 5 and 32 characters long.

Personal Details

First Name: \* 3.

Last Name: \*

Country or Location: \* 4.  
UNITED STATES



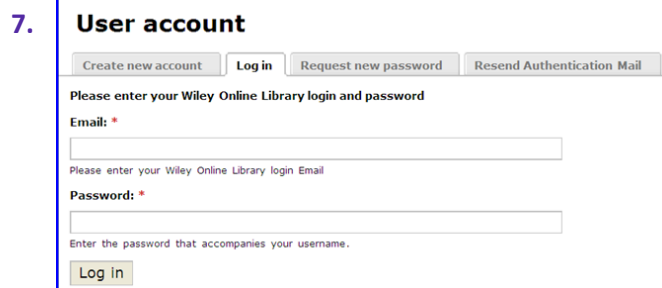
CAPTCHA

This question is for testing whether you are a human visitor and to prevent automated spam submissions.

5. moss, figure

6.

Register



**7. User account**

Create new account | Log in | Request new password | Resend Authentication Mail

Please enter your Wiley Online Library login and password

Email: \*

Please enter your Wiley Online Library login Email

Password: \*

Enter the password that accompanies your username.

Log in

注释:

主页 > 浏览

直接按目录从主页进行浏览，或点击网站上任意页面的“CATEGORIES”按钮即可浏览。

1... 点选一个分类。

2... 您可以从分类页面上浏览这个类别的新增或专题指南。

3... 您可以点击子分类菜单作进一步浏览。

4... 进入子分类菜单后您可以浏览这个分类中的指南。

5... 您可以按照日期或字母顺序将结果排序。

6... 或者通过“Current Protocols only”或“All Protocols”按钮进行筛选，“All Protocols”中还包括用户和供应商生成的指南，可供访问。

**BROWSE CATEGORIES** ▾

- ▶ Bioinformatics **1.**
- ▶ Immunology
- ▶ Protein Production, Purification, and Analysis
- ▶ Cell Biology
- ▶ Intermolecular Interactions
- ▶ Proteomics
- ▶ Cell Culture
- ▶ Laboratory Organisms and

**Bioinformatics**

**BROWSE** ▾

- ▶ Bioinformatics Fundamentals
- ▶ Biological Databases **3.**
- ▶ Cheminformatics
- ▶ DNA Analysis
- ▶ Expression Patterns
- ▶ Finding Genes
- ▶ Finding Similarities and Inferring Homologies
- ▶ Modeling Structures from Sequence
- ▶ Molecular modelling
- ▶ Phylogenetic trees
- ▶ Protein Analysis
- ▶ Proteomics
- ▶ Recognizing Functional Domains
- ▶ RNA Analysis

RSS Feed

**RECENTLY ADDED** **2.**

- ▶ Exome™ Horizon – Complete and Unified Bioinformatics learning platform
- ▶ Example of Use of TaqMan Real-Time RT-PCR to Analyze Bacterial Gene Transcript Levels: Haemophilus influenzae
- ▶ The UCSC Genome Browser: What Every Molecular Biologist Should Know
- ▶ The Importance of Biological Databases in Biological Discovery
- ▶ An Introduction to Sequence Similarity (“Homology”) Searching

**FEATURED PROTOCOLS** **2.**

- ▶ Searching NCBI Databases Using Entrez
- ▶ Selection of a Platform for Mutation Detection

**Bioinformatics**

**DNA Analysis** **4.**

1 - 10 of 30 1 2 3 > >>

**5.** SORT BY: **ALPHABETICAL** DATE **6.** FILTER BY: **All Protocols** Current Protocols only

---

**Assays for DNA Damage**  
Print Publication Date: November, 1999  
Source: Current Protocols in Toxicology

---

**Assembling Genomic DNA Sequences with PHRAP**  
Print Publication Date: March, 2007  
Source: Current Protocols in Bioinformatics

---

**Computer Manipulation of DNA and Protein Sequences**  
Print Publication Date: April, 1995  
Source: Current Protocols in Molecular Biology

注释:

主页 > 搜索

直接从主页或网站中的任意页面搜索具体词汇或指南。

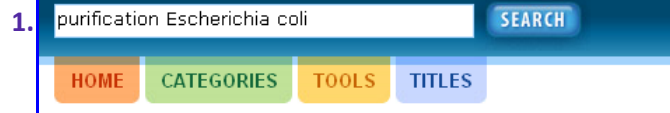
1...输入搜索词汇。

2...您可以在搜索结果页面上将结果按照日期或字母顺序排列。

3...仅通过“Current Protocols”或“All Protocols”按钮进行筛选，“All Protocols”中还包括用户和供应商生成的指南，可供访问。

4...通过分类或“出版物”作进一步搜索。

5...点击标题即可浏览指南。



**Search Results**

purification Escherichia coli

2. **SORT BY**

- ▶ Relevance
- ▶ Date
- ▶ Alphabetical

3. **REFINE THESE RESULTS**

All protocols [What's this?](#)  
 Current Protocols only

9 matches found for **purification Escherichia coli**

**Preparation of Soluble Proteins from Escherichia coli**  
**Author(s):** Paul T. Wingfield  
**Publication Date:** August, 2005  
**Source:** Current Protocols in Protein Science

**Selection of Escherichia coli Expression Systems**  
**Author(s):** Alain Bernard, Mark Payton  
**Publication Date:** June, 1995  
**Source:** Current Protocols in Protein Science

**IN CATEGORIES:**

- ▶ Cell Culture (2)
- ▶ Microbiology (1) **4.**
- ▶ Molecular Biology (5)

**IN PUBLICATIONS:**

- ▶ Current Protocols in Molecular Biology (1)
- ▶ Current Protocols in Protein Science (8)

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

**Author(s):** Paul T. Wingfield, Ira Palmer, Shu-Mei Liang

**Publication Date:** June, 1995

**Source:** Current Protocols in Protein Science

注释:

浏览内容

currentprotocols.com上的所有内容均是免费提供的。指南的全文在[Wiley InterScience](http://Wiley InterScience)的网站上，且可供订阅。

- 1... 您可以在此浏览摘要。
- 2... 目录。
- 3... 材料列表 — 包括供应商的链接（位于列表底部）。
- 4... 图示
- 5... 点击图示或“View Image（浏览图片）”链接可放大。
- 6... 在图片上点击鼠标右键即可保存或复制图片。
- 7... 浏览引用文献列表。
- 8... 在 PDF 上点击或点击 HTML 链接即可浏览全文。



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

PEER REVIEWED

Paul T. Wingfield<sup>1</sup>, Ira Palmer<sup>1</sup>, Shu-Mei Liang<sup>2</sup>

<sup>1</sup>National Institutes of Health, Bethesda, Maryland  
<sup>2</sup>North American Vaccine Corp., Beltsville, Maryland

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

**USER RATINGS**

Easy to Follow  
★★★★☆  
Your rating: None (2 votes)

Achieved Expected Results  
★★★★☆  
Your rating: None (1 vote)

Overall Rating  
★★★★☆  
Your rating: None (1 vote)

[Add your comments ▶](#)

---

**8.**

GO TO THE FULL TEXT: [PDF](#) or [HTML](#) at Wiley Interscience

Are you the author of this protocol? [Login](#) or [register](#) and return to this page.  
Learn more about the author(s): Paul Wingfield

**ABSTRACT 1.**

2. TABLE OF CONTENTS

3. MATERIALS

4. FIGURES

7. LITERATURE CITED

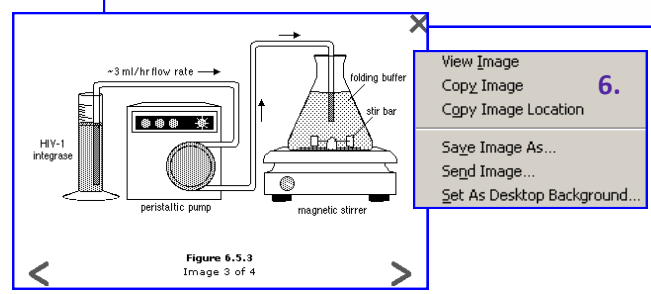
AUTHOR NOTES

**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 "foldit" buffer before folding and the protein is folded

NOTE: All steps are carried at 4°C unless otherwise stated.  
Looking for materials? [Suppliers Guide 3.](#)

**Figure 6.5.3**  
Setup for folding of HIV-1 integrase by dilution into buffer.  
**5. View Image**



注释:



内容概览 > 更多

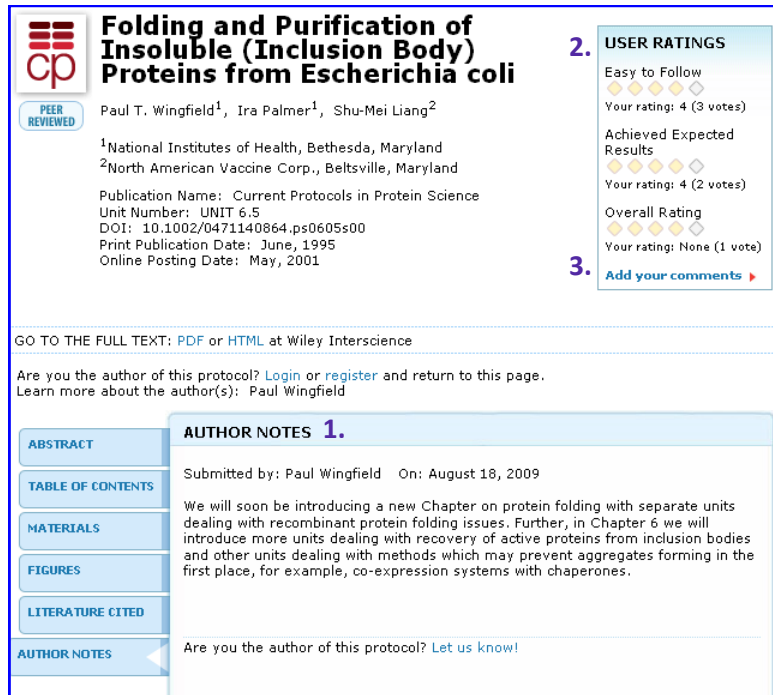
currentprotocols.com有两个特殊功能可提升实验室指南的讨论和流通性能。

1... “Author Notes (作者注释)” 部分允许作者添加注释和评论 — 使内容和讨论与现实问题紧密相连。

2... 您也可以对指南作出评价，

3... 您还可以提出疑问，提供建议、方法或改善措施，与实验室指南社区实现共享。

您不需要注册或订阅即可使用以上功能。



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

Paul T. Wingfield<sup>1</sup>, Ira Palmer<sup>1</sup>, Shu-Mei Liang<sup>2</sup>

<sup>1</sup>National Institutes of Health, Bethesda, Maryland  
<sup>2</sup>North American Vaccine Corp., Beltsville, Maryland

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

**2. USER RATINGS**

Easy to Follow  
Your rating: 4 (3 votes)

Achieved Expected Results  
Your rating: 4 (2 votes)

Overall Rating  
Your rating: None (1 vote)

**3. Add your comments**

GO TO THE FULL TEXT: [PDF](#) or [HTML](#) at Wiley Interscience

Are you the author of this protocol? [Login](#) or [register](#) and return to this page.  
Learn more about the author(s): Paul Wingfield

**ABSTRACT**

**TABLE OF CONTENTS**

**MATERIALS**

**FIGURES**

**LITERATURE CITED**

**AUTHOR NOTES**

**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.

Are you the author of this protocol? [Let us know!](#)

**3. Looking for Answers?**  
Do you have tips, tricks, or improvements to share?

**JOIN THE CONVERSATION**

**YOUR NAME:** Anonymous

**E-MAIL:**   
The content of this field is kept private and will not be shown publicly.

**HOME PAGE:**

**SHARE YOUR COMMENTS:**

**B I**

**PREVIEW** **SUBMIT COMMENT**

注释:

主页 > 视频指南

currentprotocols.com提供免费的视频指南。

1...您可以从主页浏览专题视频指南。

2...点击“See a list of all videos（查看所有视频列表）”链接，即可查看所有可看视频。

3...点击图片或标题即可浏览。

4...您可以像文本指南一样提出疑问，提供建议、方法或改善措施。

**1. Video protocols available!**  
Watch peer-reviewed, high quality step-by-step video protocols for top lab procedures!  
**2.** [See a list of all videos ▶](#)



**FEATURED VIDEO**  
Phase Contrast and Differential Interference Contrast (DIC) Microscopy

**Current Protocols Videos**



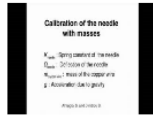
**3. Alternate Aphid Feeding Chamber**

Duration: 1:50  
[Play Video ▶](#)



**Aphid Feeding Chamber**

Duration: 2:06  
[Play Video ▶](#)



**Calibration of the needle with masses**

Duration: 0:29  
[Play Video ▶](#)



**Chromosome**

Duration:   
[Play Video ▶](#)



**Coating glass**

Duration:   
[Play Video ▶](#)



**Counting**

Duration:   
[Play Video ▶](#)



Alternate Aphid Feeding Chamber, CPMC UNIT 16B.1  
Duration: 1:50  
Video Seq: 2

**RELATED PROTOCOLS:**  
[Aphid Transmission of Plant Viruses](#)

**4. Looking for Answers?**  
Do you have tips, tricks, or improvements to share?  
[JOIN THE CONVERSATION](#)

注释:

主页 > 工具和计算器

currentprotocols.com上的工具和计算器均可免费使用，并可提供在线即时结果。

1...您可以通过“TOOLS”按钮，和所有页面右边链接到 TOOLS & CALCULATORS.

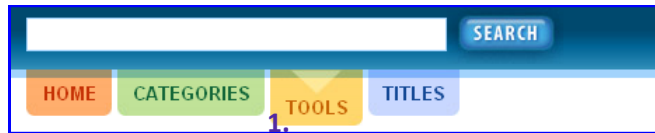
2...点击工具或计算器。

以下是使用“Buffer Calculator（缓冲液计算器）”的样例。

3...从下拉式菜单中选择缓冲液。

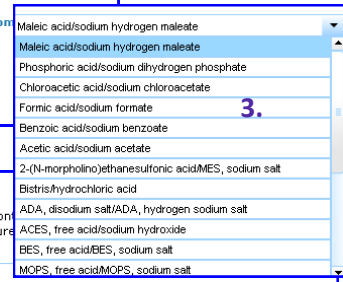
4...调节得到浓度、体积、pH值和温度。

5...结果将在下文显示。



### Tools and Calculators

- ▶ Bacterial Media Recipes Calculator
- ▶ Buffer Calculator **2.**
- ▶ Colorimetric Assay Calculation Tool
- ▶ Common Laboratory Recipes Calculator
- ▶ DNA-Protein Translator Tool
- ▶ DNA/RNA/Protein Molecular Weight Calculator
- ▶ G-Force/RPM Conversion Tool
- ▶ Hemacytometer Calculation Tool
- ▶ ImageJ Java Applet
- ▶ NEBcutter v. 2.0
- ▶ Polyacrylamide Gel Recipes Calculator
- ▶ Primer3Plus
- ▶ Radioactive Decay Calculator for Isotopes Commonly Used in Bio
- ▶ Solution Concentration Calculator
- ▶ Spectrophotometric Measurement of Nucleic Acids Calculator
- ▶ Units of Measurement Conversion Tool



### Buffer Calculator

Provides recipes for the preparation of buffers over a concentration range of 0.001 to 1000 mol/l. Enables scaling for volume and correction for temperature. Lists 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

注释:

主页 > MyCP.com

保存和整理您的最爱收藏，上传您自己的指南，与实验室指南社区实现共享。访问此功能前必须注册和登录。

1... 登录

2... 登录

currentprotocols.com 后您可以在右边看到最近浏览过的指南列表。

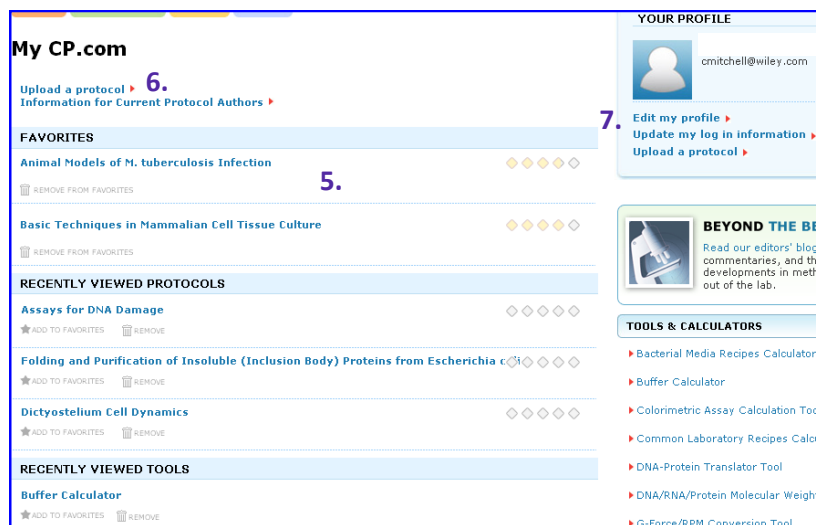
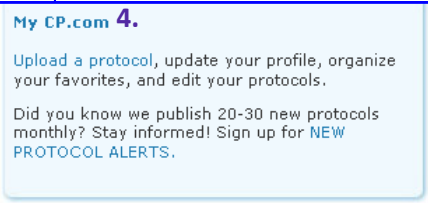
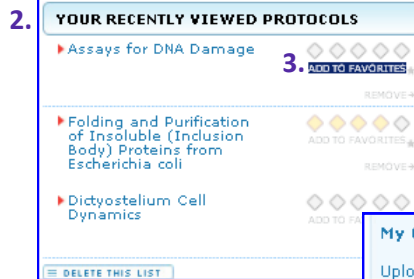
3... 可将此指南快速添加到您的最爱收藏中。

4... 点击 MyCP.com 链接即可整理您的最爱收藏，编辑您的个人资料，上传指南。

5... 从您的最爱收藏列表中添加或删除指南和工具。

6... 上传您自己的指南。

7... 编辑您的个人资料和登录信息。



注释:

主页 > MyCP.com > 上传指南

上传您自己的指南，与实验室指南社区实现共享。

1...点击“Upload a protocol（上传指南）”链接

2...您可以在“Share Your Protocol（分享您的指南）”屏幕上找到用户指南和供应商指南的提交说明。

3...点击“Publish your Protocol（发布您的指南）”，上传您的指南。

4...填写标题，选择分类。

5...填写作者详细信息。

6...添加摘要，上传您的指南文件。

7...准备就绪后点击“Save & Preview（保存并与预览）”按钮。

您上传并保存了指南以后，指南会提交给我们的编辑团队审核。审核后指南即可公布浏览。

### My CP.com

Upload a protocol **1.**  
Information for Current Protocol Authors

### Share Your Protocol

SUBMISSION GUIDE: USER PROTOCOLS **2.**  
SUBMISSION GUIDE: SUPPLIER PROTOCOLS  
PUBLISH YOUR PROTOCOL (REGISTRATION REQUIRED) **3.**

Your protocol will be available to all users of this website for as long as you wish.

Create content

### Create PDF Protocol

Title: \*

**4.**

CATEGORIES \*

Categories: \*

<none>

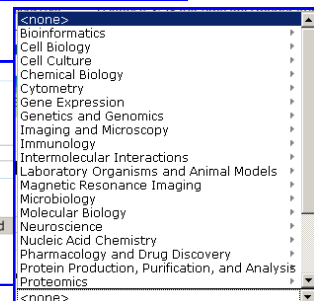
Add

SUPPLIER/AUTHOR DETAILS

All selections

PROTOCOL INFO \*

Nothing has been selected.



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

Abstract: \*

**6.**

Path:

150 word maximum length

PDF File: \*

Browse... Upload

Maximum Filesize: 20 MB  
Allowed Extensions: .pdf

PROTOCOL SUBMISSION STATUS

Draft (viewable by you only)

Submitted for CP Editorial Approval (publicly viewable after approval)

Save & Preview **7.**

注释: