APPENDICES

APPENDIX A

Serum Protein Estimation Results

Serum protein content in normotensive rat.

Sample	Protein content (µg)
NR 1	315.33 ± 0.09
NR 2	164.00 ± 0.07
NR 3	220.88 ± 0.08

Serum protein content in nontreated SHRs.

Sample	Protein content (µg)
SHR 1	113.95 ± 0.04
SHR 2	114.80 ± 0.05
SHR 3	52.13 ± 0.01
SHR 4	97.63 ± 0.01

Serum protein content in SHR treated with SF extract.

Sample	Protein content (µg)
SHR-SF 1	208.29 ± 0.12
SHR-SF 2	273.41 ± 0.12
SHR-SF 3	343.76 ± 0.08
SHR-SF 4	204.27 ± 0.08

Serum protein content in SHR treated with BL extract.

Sample	Protein content (µg)
SHR-BL 1	226.77 ± 0.02
SHR-BL 2	123.97 ± 0.03
SHR-BL 3	117.31 ± 0.10
SHR-BL 4	258.38 ± 0.13

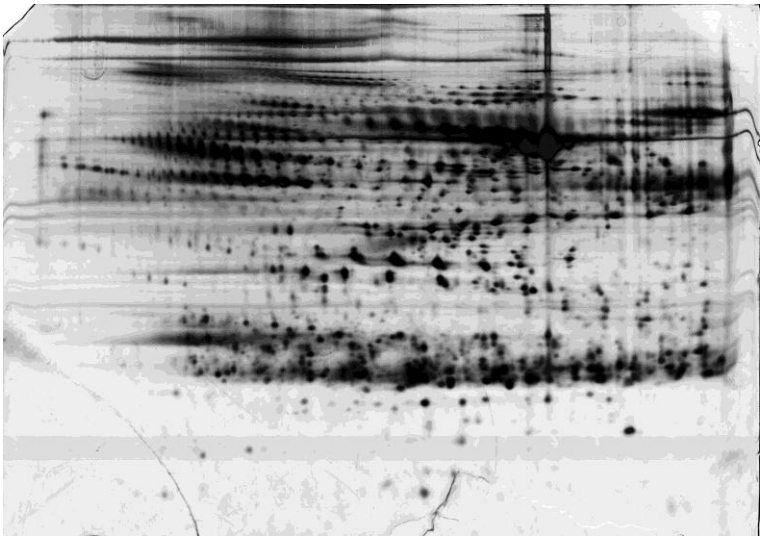
Serum protein content in SHR treated with Captopril.

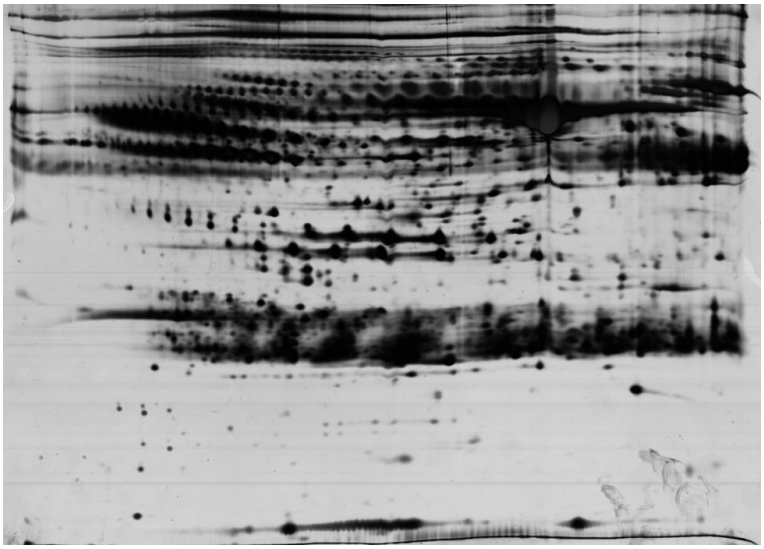
Sample	Protein content (µg)
SHR-Cp 1	377.71 ± 0.03
SHR-Cp 2	134.40 ± 0.02
SHR-Cp 3	117.87 ± 0.05
SHR-Cp 4	301.24 ± 0.07

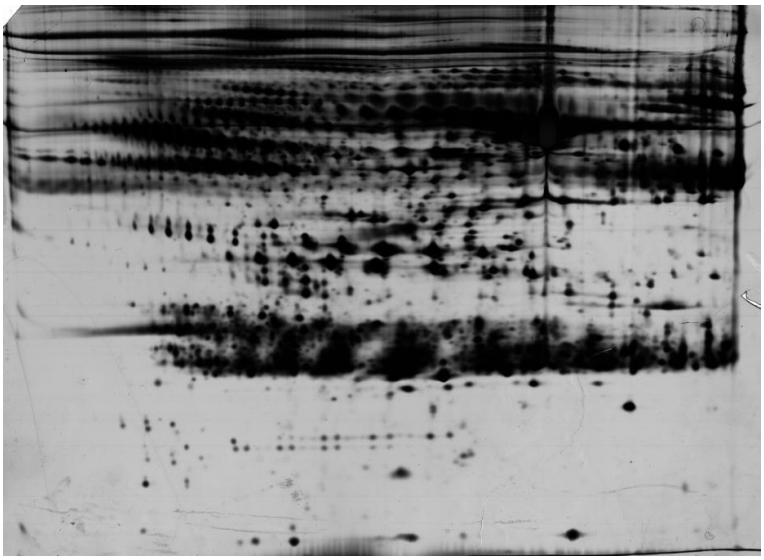
APPENDIX B

Representative 2-DE gels

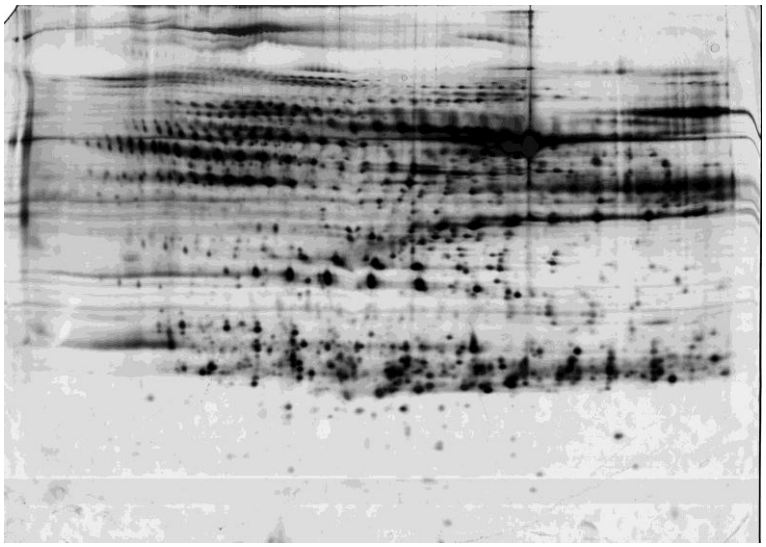
Normotensive rats (NR/SD)

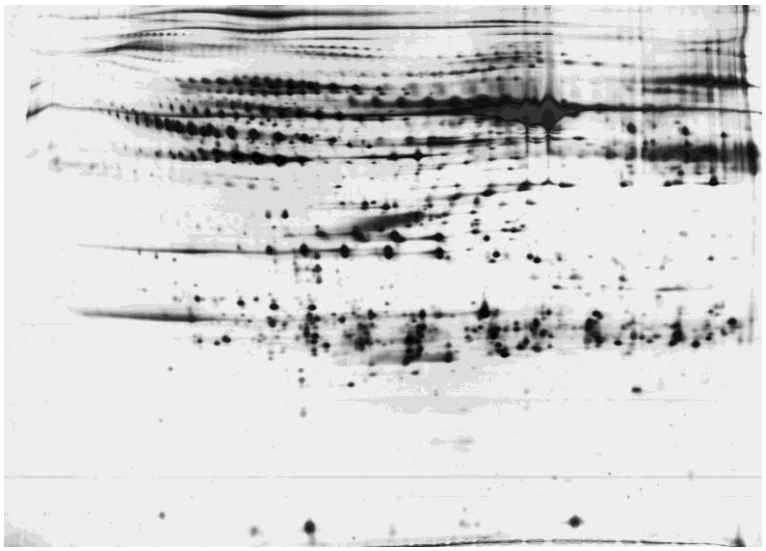


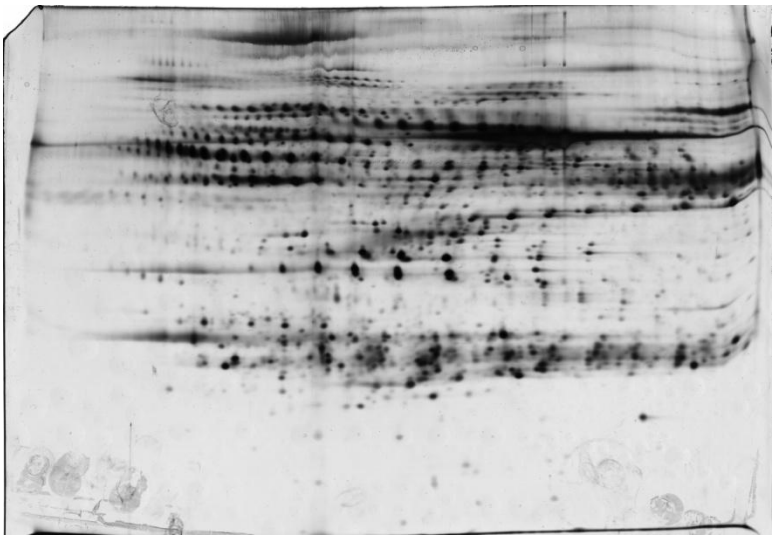




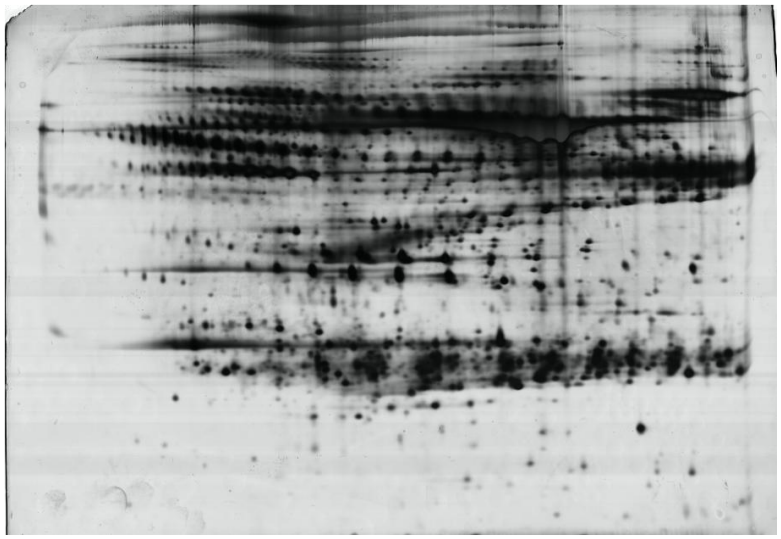
SHR RATS

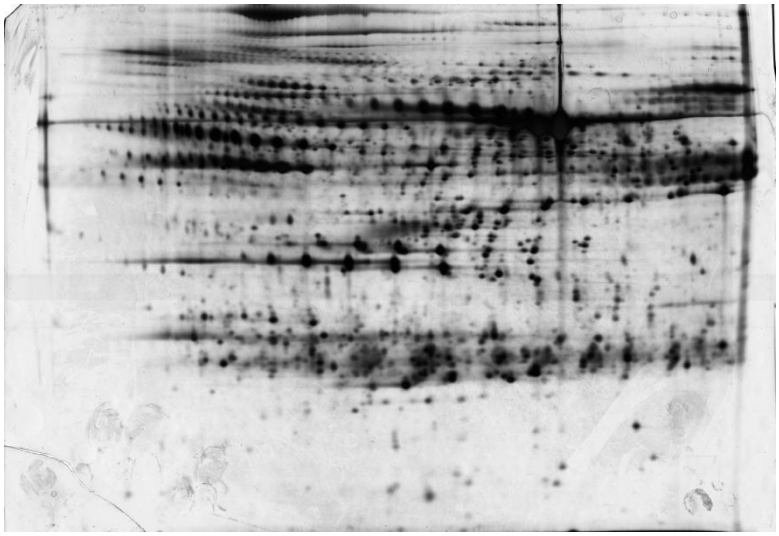


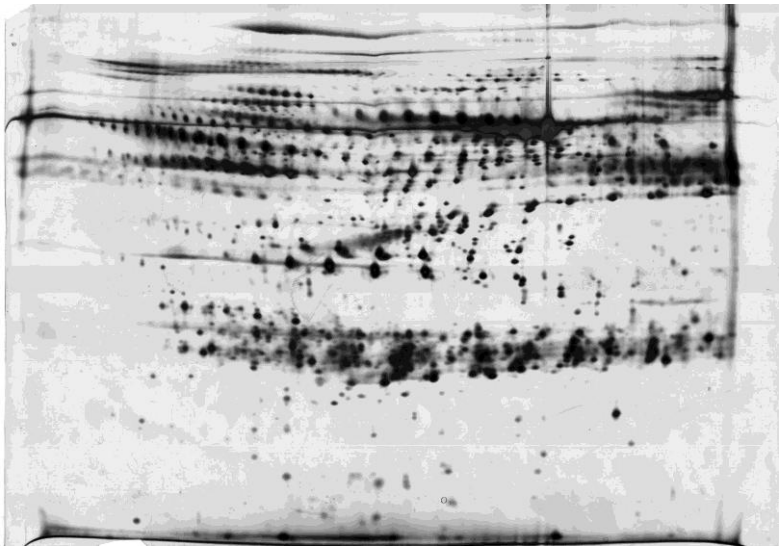




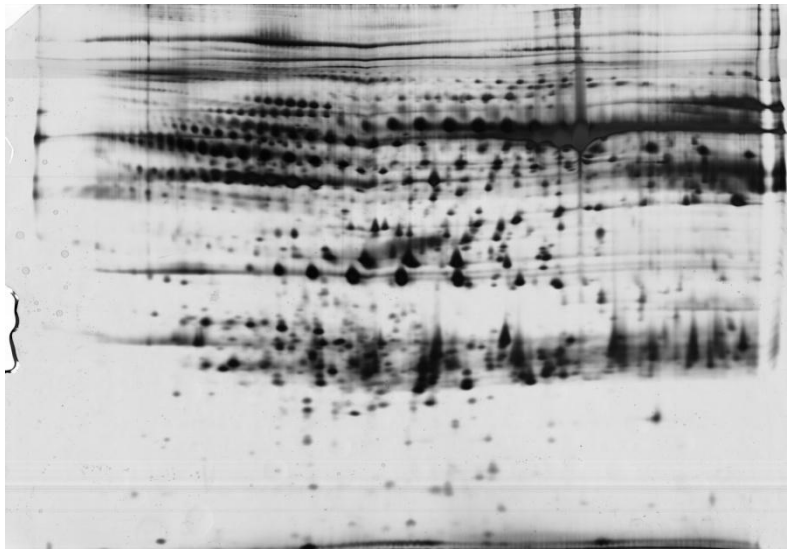
SHR-SF

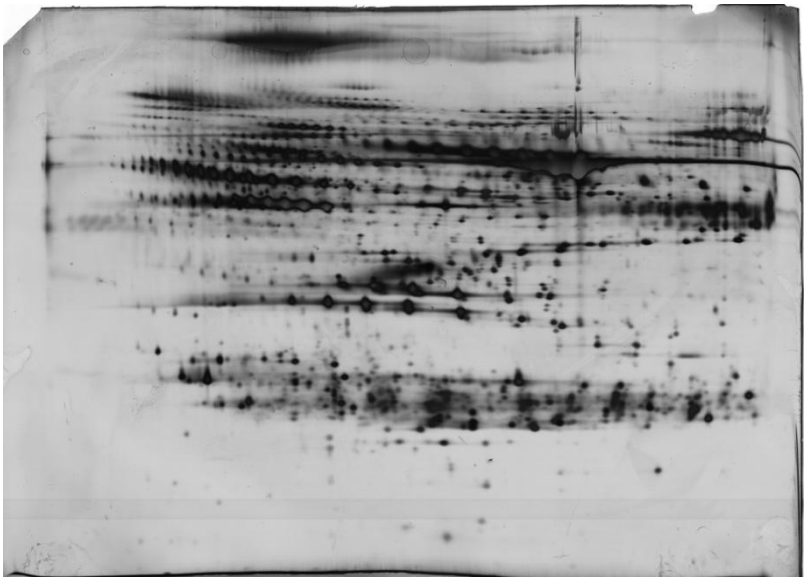


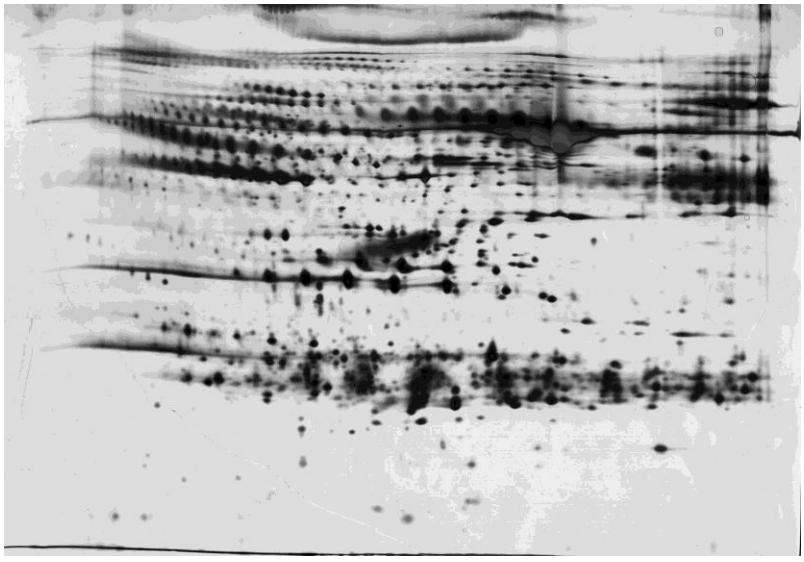




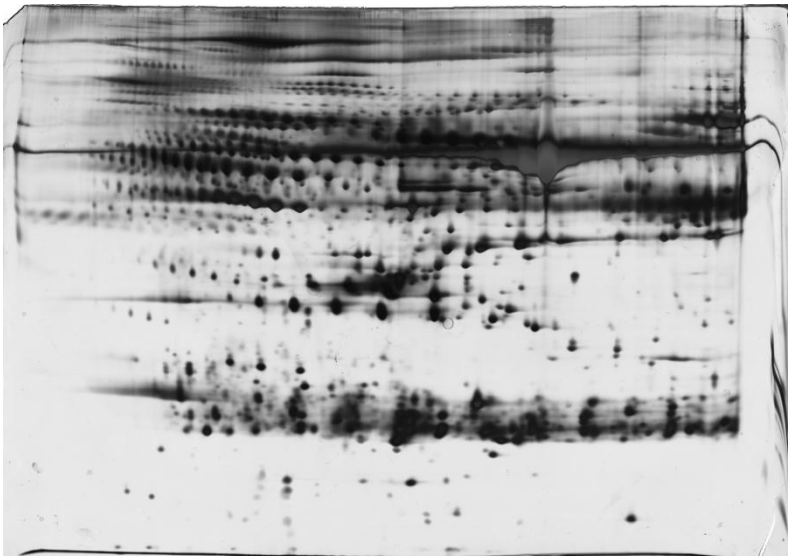
SHR-BL

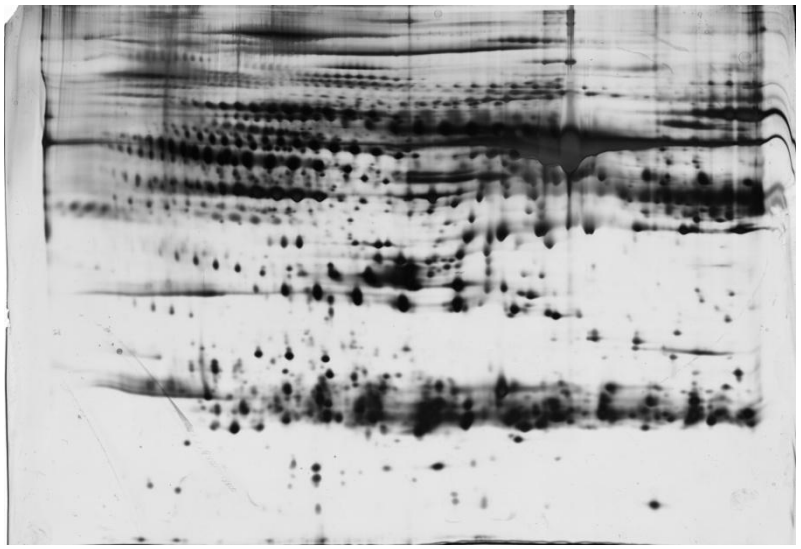


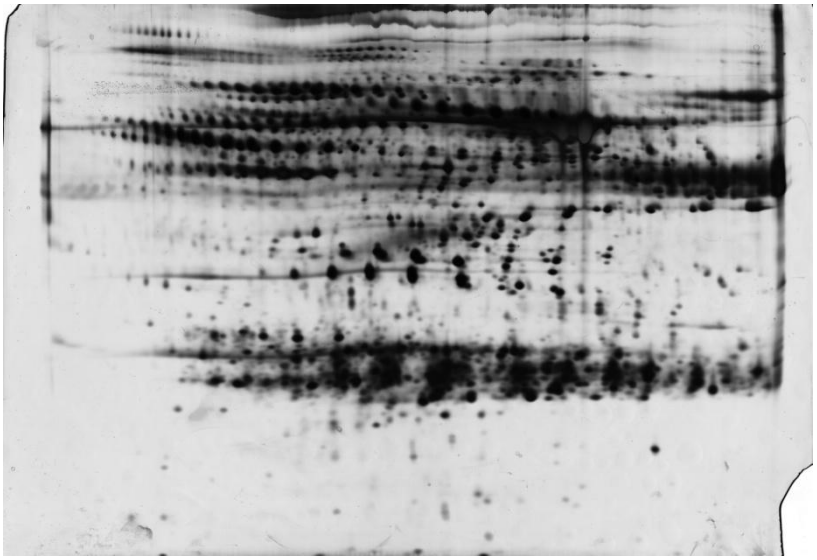




SHR-Cp

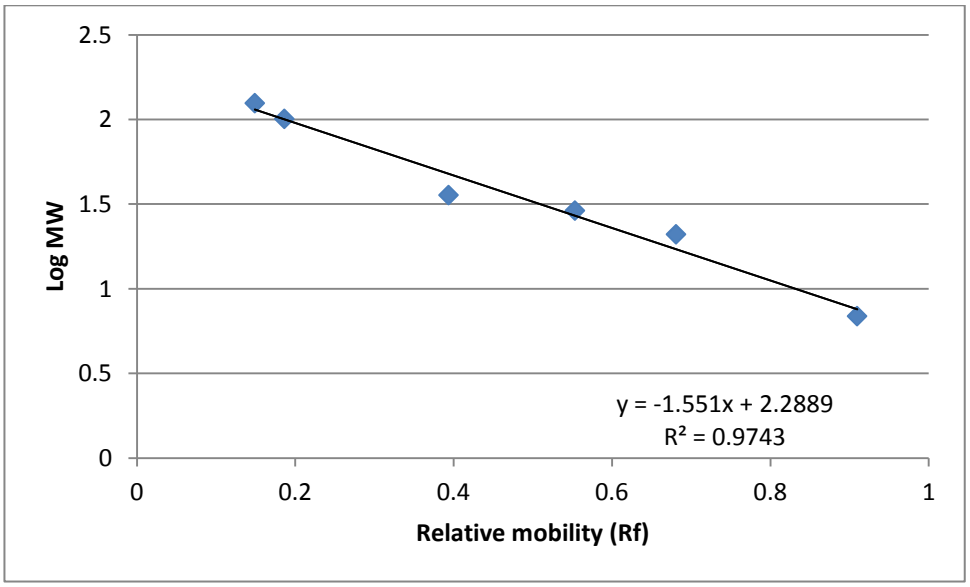






APPENDIX C

2-DE gel calibration curve



APPENDIX D

Manufacturer protocols (protein estimation)

INSTRUCTIONS

BCA Protein Assay Reagent Kit

23225 23227

1296w

PIERCE
a brand of PERBIO
3747 N. Meridian Road
P.O. Box 117
Rockford, IL 61105

Number	Description
23225	**BCA Protein Assay Reagent Kit**, sufficient reagents for 500 test tube or 5,000 microplate assays
23227	**BCA Protein Assay Reagent Kit**, sufficient reagents for 250 test tube or 2,500 microplate assays
	Kit Contents:
23228	**BCA Reagent A**, 500 ml, containing sodium carbonate, sodium bicarbonate, bicinchoninic acid and sodium tartrate in 0.1 M sodium hydroxide (Product No. 23225 contains 2 x Product No. 23228)
23224	**BCA Reagent B**, 25 ml, containing 4% cupric sulfate
23209	**Albumin Standard Ampules, 2 mg/ml**, 10 x 1 ml ampules containing bovine serum albumin (BSA) at a concentration of 2.0 mg/ml in 0.9% saline and 0.05% sodium azide

Storage: Upon arrival store at room temperature. Product shipped at ambient temperature.

Note: If either Reagent A or Reagent B precipitates upon shipping in cold weather or during long-term storage, dissolve precipitates by gently warming and stirring solution. Discard any kit reagent that shows discoloration or evidence of microbial contamination.

This product is guaranteed for one year from the date of purchase when handled and stored properly.

Table of Contents

Introduction

The Pierce BCA Protein Assay is a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantitation of total protein. This method combines the well-known reduction of Cu^{+2} to Cu^{+1} by protein in an alkaline medium (the biuret reaction) with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{+1}) using a unique reagent containing bicinchoninic acid.[1] The purple-colored reaction product of this assay is formed by the chelation of two molecules of BCA with one cuprous ion. This water-soluble complex exhibits a strong absorbance at 562 nm that is nearly linear with increasing protein concentrations over a broad working range (20–2,000 µg/ml). The BCA method is not a true end-point method; i.e., the final color continues to develop. However, following incubation, the rate of continued color development is sufficiently slow to allow large numbers of samples to be assayed together.

Telephone: 800-8-PIERCE (800-874-3723) or 815-968-0747 • Fax: 815-968-7316 or 800-842-5007
www.piercenet.com • Customer Service: cs@piercenet.com • Technical Assistance: ta@piercenet.com

The macromolecular structure of protein, the number of peptide bonds and the presence of four particular amino acids (cysteine, cystine, tryptophan and tyrosine) are reported to be responsible for color formation with BCA.[2] Studies with di-, tri- and tetrapeptides suggest that the extent of color formation caused by more than the mere sum of individual color-producing functional groups.[2] Accordingly, protein concentrations generally are determined and reported with reference to standards of a common protein such as bovine serum albumin (BSA). A series of dilutions of known concentration are prepared from the protein and assayed alongside the unknown(s) before the concentration of each unknown is determined based on the standard curve. If precise quantitation of an unknown protein is required, it is advisable to select a protein standard that is similar in quality to the unknown; for example, a bovine gamma globulin (BGG) standard (see Related Pierce Products) may be used when assaying immunoglobulin samples.

Two assay procedures are presented. Of these, the Test Tube Procedure requires a larger volume (0.1 ml) of protein sample; however, because it uses a sample to working reagent ratio of 1:20 (v/v), the effect of interfering substances is minimized. The Microplate Procedure affords the sample handling ease of a microplate and requires a smaller volume (10-25 µl) of protein sample; however, because the sample to working reagent ratio is 1:8 (v/v), it offers less flexibility in overcoming interfering substance concentrations and obtaining low levels of detection.

Preparation of Standards and Working Reagent (required for both assay procedures)

A. Preparation of Diluted Albumin (BSA) Standards

Use Table 1 as a guide to prepare a set of protein standards. Dilute the contents of one Albumin Standard (BSA) ampule into several clean vials, preferably using the same diluent as your sample. Each 1 ml ampule of 2.0 mg/ml Albumin Standard is sufficient to prepare a set of diluted standards for either working range suggested in Table 1. There will be sufficient volume for three replications of each diluted standard.

Table 1: Preparation of Diluted Albumin (BSA) Standards

Dilution Scheme for Standard Test Tube Protocol and Microplate Procedure (Working Range = 20–2,000 µg/ml)

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	0	300 µl of Stock	2,000 µg/ml
B	125 µl	375 µl of Stock	1,500 µg/ml
C	325 µl	325 µl of Stock	1,000 µg/ml
D	175 µl	175 µl of vial B dilution	750 µg/ml
E	325 µl	325 µl of vial C dilution	500 µg/ml
F	325 µl	325 µl of vial E dilution	250 µg/ml
G	325 µl	325 µl of vial F dilution	125 µg/ml
H	400 µl	100 µl of vial G dilution	25 µg/ml
I	400 µl	0	0 µg/ml = Blank

Dilution Scheme for Enhanced Test Tube Protocol (Working Range = 5–250 µg/ml)

Vial	Volume of Diluent	Volume and Source of BSA	Final BSA Concentration
A	700 µl	100 µl of Stock	250 µg/ml
B	400 µl	400 µl of vial A dilution	125 µg/ml
C	450 µl	300 µl of vial B dilution	50 µg/ml
D	400 µl	400 µl of vial C dilution	25 µg/ml
E	400 µl	100 µl of vial D dilution	5 µg/ml
F	400 µl	0	0 µg/ml = Blank

B. Preparation of the BCA Working Reagent (WR)

1. Use the following formula to determine the total volume of WR required:

   (# standards + # unknowns) x (# replicates) x (volume of WR per sample) = total volume WR required

   Example: for the Standard Test Tube Protocol with 3 unknowns and 2 replicates of each sample:

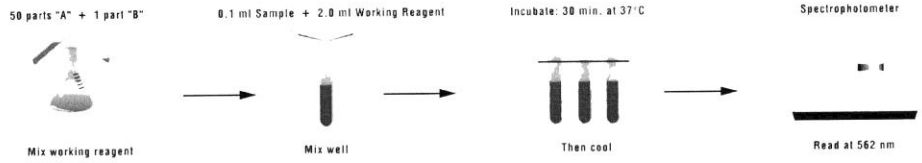
   (9 standards + 3 unknowns) x (2 replicates) x (2 ml) = 48 ml WR required

   Note: 2.0 ml of the WR is required for each sample in the Test Tube Procedure, while only 200 µl of WR reagent is required for each sample in the Microplate Procedure.

2. Prepare WR by mixing 50 parts of BCA Reagent A with 1 part of BCA Reagent B (50:1, Reagent A:B). For the above example, combine 50 ml of Reagent A with 1 ml of Reagent B.

   Note: When Reagent B is first added to Reagent A, a turbidity is observed that quickly disappears upon mixing to yield a clear, green WR. Prepare sufficient volume of WR based on the number of samples to be assayed. The WR is stable for several days when stored in a closed container at room temperature (RT).

Procedure Summary (Test Tube Procedure, Standard Protocol)



Test Tube Procedure (Sample to WR ratio = 1:20)

1. Pipette 0.1 ml of each standard and unknown sample replicate into an appropriately labeled test tube.
2. Add 2.0 ml of the WR to each tube and mix well.
3. Cover and incubate tubes at selected temperature and time:
   - Standard Protocol: 37°C for 30 minutes (working range = 20-2,000 µg/ml)
   - RT Protocol: RT for 2 hours (working range = 20-2,000 µg/ml)
   - Enhanced Protocol: 60°C for 30 minutes (working range = 5-250 µg/ml)

   Notes:

   - Increasing the incubation time or temperature increases the net 562 nm absorbance for each test and decreases both the minimum detection level of the reagent and the working range of the protocol.
   - Use a water bath to heat tubes for either Standard (37°C incubation) or Enhanced (60°C incubation) Protocol. Using a forced-air incubator can introduce significant error in color development because of uneven heat transfer.
4. Cool all tubes to RT.
5. With the spectrophotometer set to 562 nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples within 10 minutes.

   Note: Because the BCA Assay does not reach a true end point, color development will continue even after cooling to RT. However, because the rate of color development is low at RT, no significant error will be introduced if the 562 nm absorbance measurements of all tubes are made within 10 minutes of each other.
6. Subtract the average 562 nm absorbance measurement of the Blank standard replicates from the 562 nm absorbance measurement of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average Blank-corrected 562 nm measurement for each BSA standard vs. its concentration in µg/ml. Use the standard curve to determine the protein concentration of each unknown sample.

Microplate Procedure (Sample to WR ratio = 1:8)

1. Pipette 25 µl of each standard or unknown sample replicate into a microplate well (working range = 20-2,000 µg/ml).

   Note: If sample size is limited, 10 µl of each unknown sample and standard can be used (sample to WR ratio = 1:20). However, the working range of the assay in this case will be limited to 125-2,000 µg/ml.
2. Add 200 µl of the WR to each well and mix plate thoroughly on a plate shaker for 30 seconds.
3. Cover plate and incubate at 37°C for 30 minutes.

XXX

4. Cool plate to RT.
5. Measure the absorbance at or near 562 nm on a plate reader.

   Notes:
   - Wavelengths from 540-590 nm have been used successfully with this method.
   - Because plate readers use a shorter light path length than cuvette spectrophotometers, the Microplate Procedure requires a greater sample to WR ratio to obtain the same sensitivity as the standard Test Tube Procedure. If higher 562 nm measurements are desired, increase the incubation time to 2 hours.
   - Increasing the incubation time or ratio of sample volume to WR increases the net 562 nm measurement for each well and lowers both the minimum detection level of the reagent and the working range of the assay. As long as all standards and unknowns are treated identically, such modifications may be useful.
6. Subtract the average 562 nm absorbance measurement of the Blank standard replicates from the 562 nm measurements of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average Blank-corrected 562 nm measurement for each BSA standard vs. its concentration in µg/ml. Use the standard curve to determine the protein concentration of each unknown sample.

   Note: If using curve-fitting algorithms associated with a microplate reader, a four-parameter (quadratic) or best-fit curve will provide more accurate results than a purely linear fit. If plotting results by hand, a point-to-point curve is preferable to a linear fit to the standard points.

Troubleshooting

Problem	Possible Cause	Solution
No color in any tubes	Sample contains a copper chelating agent	Dialyze, desalt, or dilute sample Increase copper concentration in working reagent (e.g., use 50:2, Reagent A:B) Remove interfering substances from sample using Product No. 23215
Blank absorbance is OK, but standards and samples show less color than expected	Strong acid or alkaline buffer, alters working reagent pH	Dialyze, desalt, or dilute sample
	Color measured at the wrong wavelength	Measure the absorbance at 562 nm
Color of samples appear darker than expected	Protein concentration is too high	Dilute sample
	Sample contains lipids or lipoproteins	Add 2% SDS to the sample to eliminate interference from lipids[3] Remove interfering substances from sample using Product No. 23215
All tubes (including blank) are dark purple	Buffer contains a reducing agent	Dialyze or dilute sample Remove interfering substances from sample using Product No. 23215
	Buffer contains a thiol	
	Buffer contains biogenic amines (catecholamines)	
Need to measure color at a different wavelength	Colorimeter does not have 562 nm filter	Color may be measure at any wavelength between 540 nm and 590 nm, although the slope of standard curve and overall assay sensitivity will be reduced

A. Interfering substances

Certain substances are known to interfere with the BCA Assay including those with reducing potential, chelating agents, and strong acids or bases. Because they are known to interfere with protein estimation at even minute concentrations, avoid the following substances as components of the sample buffer:

Ascorbic Acid	EGTA	Iron	Impure Sucrose
Catecholamines	Impure Glycerol	Lipids	Tryptophan
Creatinine	Hydrogen Peroxide	Melibiose	Tyrosine
Cysteine	Hydrazides	Phenol Red	Uric Acid

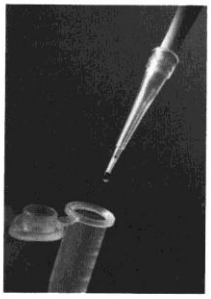
APPENDIX E

Manufacturer protocol (ZipTip)

User Guide
for Reversed-Phase

ZipTip®

Pipette Tips for Sample Preparation



MILLIPORE

MILLIPORE

Notice

PR02358, Rev. A, 02/07

Introduction

The ZipTip® pipette tip is a 10 µL pipette tip with a bed of chromatography media fixed at its end. It is intended for concentrating and purifying peptide, protein or oligonucleotide samples.

These instructions describe the use of ZipTip pipette tips containing C_{18} and C_4 reversed-phase media for desalting and concentrating peptides and proteins.

For information on concentrating and desalting oligonucleotide samples, request Millipore publication TN225.

NOTE: Because the adsorptive bed provides a slight back pressure, do not use the ZipTip pipette tip for accurate volumetric dispensing. To achieve optimal sample uptake and delivery, set the pipettor to 10 µL and attach the ZipTip pipette tip securely. Throughout the procedure, depress and release the plunger slowly to ensure optimal movement of solution through the resin bed.

Materials

The following table outlines the solutions required for use with ZipTip pipette tips containing C_{18} and C_4 media. C_{18} is offered in two bed volumes:

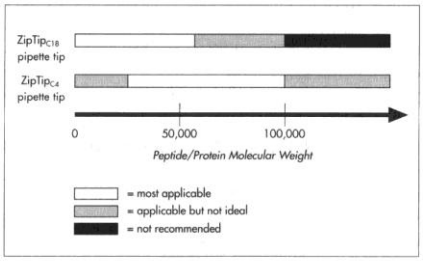
- $ZipTip_{C18}$ tips — a standard bed of 0.6 µL for sample elution in 1 to 4 µL
- $ZipTip_{\mu\text{-}C18}$ tips — a micro bed of 0.2 µL for elution in < 1 µL

The procedure also requires a compatible 10 µL pipettor. For simultaneous processing of multiple samples, Millipore recommends the Biohit Proline™ Multichannel Pipettor.

Solution	$ZipTip_{C18/\mu\text{-}C18}$ Pipette Tips	$ZipTip_{C4}$ Pipette Tips
Wetting solution	100% acetonitrile (ACN)	100% acetonitrile (ACN)
Sample preparation	Adjust sample to 0.1% trifluoroacetic acid (TFA); final sample pH should be <4	Adjust sample to 0.1% trifluoroacetic acid (TFA); final sample pH should be <4 (Optional) Guanidine-HCl 1-6M may be added.
Equilibration solution	0.1% TFA in Milli-Q® grade water	0.1% TFA in Milli-Q grade water
Wash solution	0.1% TFA in Milli-Q grade water	0.1% TFA in Milli-Q grade water
Elution solution*	0.1% TFA/50% ACN with or without matrix	0.1% TFA/50-75% ACN with or without matrix

** For electrospray, elute with 1% formic acid/50% methanol. For fractionating peptides, prepare varying concentrations of ACN/water (e.g. 5%, 10%, 20%, 30%, and 50%) with or without 0.1% TFA.*

Guidelines for Selecting $ZipTip_{C18}$ or $ZipTip_{C4}$ Pipette Tips



$ZipTip_{C18}$ pipette tips are most applicable for peptides and low molecular weight proteins, while $ZipTip_{C4}$ pipette tips are most suitable for low to intermediate molecular weight proteins. In many cases, the two devices can be used interchangeably. Because higher molecular weight proteins tend to adsorb tenaciously to hydrophobic surfaces, $ZipTip_{C4}$ pipette tips are recommended for proteins over 100,000 MW.

Procedures for Use

The following procedures describe how to prepare the sample and equilibrate the ZipTip pipette tips for sample binding, washing, and elution. See the "Materials" section for information on the appropriate solutions for your application.

Prepare the Sample

Maximum binding to the ZipTip pipette tip is achieved in the presence of TFA (0.1%) or other ion-pairing agents. Ensure that the final sample solution has a pH<4.

Optimal binding of protein to the $ZipTip_{C4}$ pipette tip may also require a chaotropic agent (e.g., guanidine-HCl at a final concentration of 1–6M). If the sample does not already contain chaotropic salts, add them a few minutes before binding. These salts will be removed during the wash step following sample binding.

Equilibrate the ZipTip Pipette Tip for Sample Binding

1. Depress pipettor plunger to a dead stop. Using the maximum volume setting of 10 µL, aspirate **wetting solution** into the tip. Dispense to waste. Repeat.
2. Aspirate **equilibration solution.** Dispense to waste. Repeat.

Bind and Wash the Peptides or Proteins

Follow these steps after equilibrating the ZipTip pipette tip:

1. Bind peptides and/or proteins to ZipTip pipette tip by fully depressing the pipette plunger to a dead stop. Aspirate and dispense the sample 7–10 cycles for maximum binding of complex mixtures.
2. Aspirate **wash solution** into tip and dispense to waste. Repeat at least once.

   NOTE: A 5% methanol in 0.1% TFA/water wash can improve desalting efficiency. Additional washing may be required for electrospray MS.

Elute the Peptides or Proteins

For $ZipTip_{C18}$ (standard bed format) and $ZipTip_{C4}$, pipette tips, dispense 1 to 4 µL of **elution solution** into a clean vial using a standard pipette tip. In the case of $ZipTip_{\mu\text{-}C18}$ (micro bed format) pipette tips, dispense 0.5 to 2 µL of elution solution into a clean vial.

CAUTION: Acetonitrile and methanol are volatile and evaporation can occur rapidly. If this occurs, add more eluant to recover sample.

Carefully, aspirate and dispense eluant through ZipTip pipette tip at least three times without introducing air into the sample. Sample recovery can be improved (at the expense of concentration) by increasing elution volume to 5 µL.

For Direct Spotting onto a MALDI-TOF MS Target

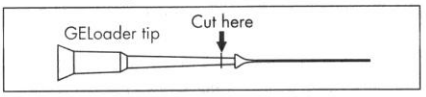
Elute with or without matrix in **elution solution.**

1. Pipette 0.5 to 4 µL of desalted-concentrated sample directly onto target by depressing plunger until appropriate volume is dispensed.
2. Save or discard the remaining sample.

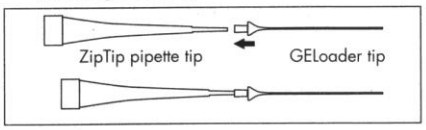
For Nanoelectrospray MS

Sample can be eluted into clean vial or, using a GELoader™ tip (Eppendorf cat. no. 0030 001 222), into a nanospray needle.

1. Cut the GELoader tip about 2–3 mm above where the tip is fused to its capillary end.



2. Before the final dispense, firmly press the cut-down GELoader tip onto the ZipTip pipette tip with a slight twisting motion.



The leak-free fit allows elution directly into a nanospray needle.

For Fractionating Peptides or Proteins

1. Pipette 1 to 3 µL of 0.1% TFA/5% acetonitrile into clean vial. Perform 3–4 aspirate-dispense cycles to elute hydrophilic peptides or proteins from tip. Use the final dispense cycle to apply peptides or proteins directly to target.
2. Wash tip immediately by aspirating 0.1% TFA/5% acetonitrile. Dispense to waste. Repeat twice.
3. Perform next step gradient (e.g. 5, 10, 20, 30 or 50% ACN) by increasing acetonitrile and repeat steps 1 and 2 until step-gradient is completed.

NOTE: Thoroughly wash the tip with respective eluant prior to increasing ACN elution to minimize peptide or protein carry-over.

Chemical Compatibility

✔ = Acceptable. Long exposures at room temperature have no significant effect.

? = Questionable. Short exposures at room temperature cause little or no damage.

X = Not recommended. Short exposure may cause permanent damage.

Reagent	ZipTip Pipette Tips
Acetic Acid (Glacial)	✔
Acetone	X
Acetonitrile (100%)	✔
Aliphatic Esters	?
Ammonium Hydroxide (5%)	✔
Benzene (100%)	X
n-Butanol (100%)	✔
Butyl Acetate	X
Chloroform (1%)	?
Dichlorobenzene (100%)	X
Dichloromethane (1%)	?
Diethanolamine (5%)	✔
Dimethyl Acetamide (100%)	X
Dimethyl Formamide (100%)	X
Dimethyl Formamide (1%)	✔
Ethanol (100%)	✔
Formic Acid (5%)	✔
Guanidine HCl (6 M)	✔
Hydrochloric Acid	✔

Chemical Compatibility, continued

Reagent	ZipTip Pipette Tips
Hydrogen Peroxide	✔
Isopropyl Alcohol (100%)	✔
Mercaptoethanol (0.1 M)	✔
Mercaptoethanol (1.0 M)	?
Methyl Alcohol (100%)	✔
Methyl Ethyl Ketone (100%)	X
Methyl Isobutyl Ketone (100%)	✔
Nitric Acid (0.1 N)	✔
Nitric Acid (1.5 N)	✔
Phenol (0.5%)	X
Phosphoric Acid (1M)	✔
Sodium Azide (1%)	✔
Sodium Hydroxide (0.5 N)	✔
Sodium Hydroxide (0.1 N)	✔
Sodium Hypochlorite (100 ppm)	✔
Sodium Hypochlorite (200 ppm)	✔
Sulfuric Acid (1%)	✔
Toluene (1%)	X
Triton® X-100 surfactant	✔
Tween® emulsifier	✔
Urea (6 M)	✔

Troubleshooting

Sample preparation problems with ZipTip$_{C18}$ (standard and micro bed formats) and ZipTip$_{C4}$ pipette tips can be divided into two categories:

- sample does not bind
- sample binds but is not recovered

The following table outlines common problems and their possible causes, and suggests procedures to solve those problems. The procedures may or may not work with your ZipTip application.

Incomplete binding is the problem most often encountered when performing sample preparation. To determine whether the problem is binding or elution, the best approach is to try the first three procedures for incomplete binding in the following table. Then, use a step-gradient approach to elution. First, elute the tip with 50% ACN in 0.1% TFA. Then, repeat the elution (on the same tip) with 75% ACN in 0.1% TFA. This method addresses both types of problems using a single sample.

If the problems persist, contact Millipore Technical Service for further suggestions.

Incomplete Binding

Possible Cause	Suggested Procedure
C_{18}/C_4 beads dewetted before sample was applied. The hydrophobic beads can de-wet in less than a minute.	After wetting with ACN, flush the tip with 0.1% TFA and leave the plug immersed in liquid until immediately before sample binding.
Sample was not sufficiently acidified with TFA. The pH should be below 4. The TFA concentration should be between 0.1–1.0%.	Spike sample with a few microliters of 0.5–1% TFA.
Sample not freely soluble.	Add Guanidine HCl to the sample to achieve a final concentration between 1–6M. Guanidine actually enhances binding by helping to wet the hydrophobic surface and reducing polypeptide secondary structure.
Sample too hydrophilic for adsorption.	Relatively few options for solving this problem. The best approach is to use a ZipTip$_{SCX}$ pipette tip instead of a reversed-phase tip.
Sample amount too low for detection.	Make sure samples are within the detection limits of the instrument. In general, a good MS signal should be obtained with 1 picomole of sample (e.g. 5 µL of a 0.2 picomole/µL solution). Substantially lower amounts can be detected if the sample is clean.

Incomplete Elution

Possible Cause	Suggested Procedure
Sample tenaciously adsorbed to the C_{18}/C_4 particles.	Increase acetonitrile content of desorption solution to a maximum of 75–90% ACN (v/v) in 0.1% TFA.
Sample not freely soluble in ACN.	Decrease ACN concentration to 20–40% in a 0.1% TFA or suitable ion-pairing agent.

Specifications

Materials of Construction

Pipette tip: Polypropylene

Media: C_{18}: spherical silica, 15 µm, 200Å pore size

C_4: spherical silica, 15 µm, 300Å pore size

Tip volume 10 µL

Adsorptive bed

C_{18}*(standard bed format):* 0.6 µL

C_{18}*(micro bed format):* 0.2 µL

C_4: 0.6 µL

Length 31 mm (1.22 in.)

Capacity

(when used with saturating amounts of analyte):

C_{18}*(standard bed format):* ≥ 1.0 µg; typically 5.0 µg

C_{18}*(micro bed format):* typically 2.0 µg

C_4: ≥ 0.5 µg; typically 3.3 µg

Max. Temperature 70 °C

Min. Temperature 4 °C

Working pH Range 2 to 13

Ordering Information

ZipTip Pack	Resin Type	Catalogue Number
8 pack, resealable	C_{18}(standard bed)	ZTC 18S 008
	C_{18}(micro bed)	ZTC 18M 008
	C_4	ZTC 04S 008
96 pack, tip rack	C_{18}(standard bed)	ZTC 18S 096
	C_{18}(micro bed)	ZTC 18M 096
	C_4	ZTC 04S 096
960 pack, 10 × 96 tip rack	C_{18}(standard bed)	ZTC 18S 960
	C_{18}(micro bed)	ZTC 18M 960
	C_4	ZTC 04S 960

For a complete listing of available ZipTip chemistries, visit www.millipore.com/ziptip or contact your local Millipore office.

Technical Assistance

For more information, contact the Millipore office nearest you. In the U.S., call **1-800-MILLIPORE** (1-800-645-5476). Outside the U.S., see your Millipore catalogue for the phone number of the office nearest you or go to our web site at www.millipore.com/offices for up-to-date worldwide contact information. You can also visit the tech service page on our web site at www.millipore.com/ techservice.

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