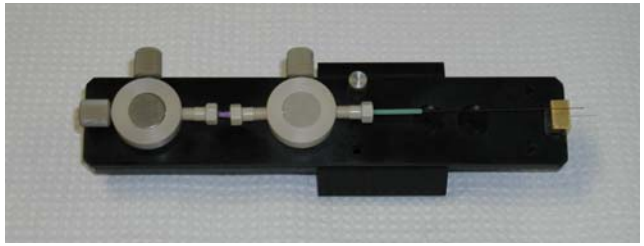
MICHROM

Michrom BioResources, Inc. offers a wide range of innovative products for HPLC and LC/MS analysis of biological and pharmaceutical samples. Products include: HPLC instrumentation, MS instrumentation, LC/MS instrumentation, Autosamplers, Fraction Collectors, Detectors, Columns, Traps, Standards, Tryptic Digests, Fittings, Tubing, HPLC Supplies, and much more.

Need More Sensitivity?

Call for details on our new NanoTrap Stage for attomole - picomole peptide mapping.



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MICHROM Bioresources, Inc.
Expanding The Vision For Life Science Analysis

Guide to Trap Cartridge Care and Use

General Tips for Michrom Trap Cartridges

Michrom offers a variety of trap cartridges packed with application specific HPLC column packing materials for concentration, desalting, detergent removal and protein removal from samples prior to analysis by HPLC, LC/MS, MALDI-MS, Edman sequencing and/or amino acid analysis. These cartridges can be used individually or in series to cleanup samples manually, on-line using an HPLC injector or automatically with an autosampler. These application specific cartridges are available in three sizes: capillary (CapTrap™), microbore (MicroTrap™) and macrobore (MacroTrap™), with capacities as shown in the table below.

Cartridge Type	Internal Dimensions	Bed Volume	Sample Capacity	Sample Volume	Speed of Loading*
MacroTrap™	3 x 8 mm	50 µl	200 µg	10-10000 µl	500-2000 µl/min
MicroTrap™	1 x 8 mm	5.0 µl	20 µg	1.0-1000 µl	50-200 µl/min
CapTrap™	0.5 x 2 mm	0.5 µl	2 µg	0.1-100 µl	5-20 µl/min

Samples can be cleaned up without the use of an HPLC by using a manual trap holder kit as shown below:

Samples can be cleaned up on-line using a loop/holder on an HPLC injection valve, an autosampler injection valve or a MS divert valve as shown below:

Brands of Valves Supported:

Valco
Cheminert
Rheodyne
Most HPLC Valves

Need More Sensitivity? Call for details on our new NanoTrap Stage for Attomole - picomole peptide mapping.

Part Numbers & Pricing Information

Traps:

Trap Description	Part Number	Price
SDS MacroTrap (Each)	TR1/25108/54	$ 75.00
SDS MacroTrap (6 Pack)	TR1/25109/54	$395.00
SDS MicroTrap (Each)	TR1/25108/04	$ 75.00
SDS MicroTrap (6 Pack)	TR1/25109/04	$395.00

Trap Description	Part Number	Price
NID Removal MacroTrap (Each)	TR125108/57	$ 75.00
NID Removal MacroTrap (6 Pack)	TR1/25109/57	$395.00
NID Removal MicroTrap (Each)	TR1/25108/07	$ 75.00
NID Removal MicroTrap (6 Pack)	TR1/25109/07	$395.00

Trap Description	Part Number	Price
ISRP Protein Removal MacroTrap	TR1/25108/58	$ 75.00
ISRP Protein Removal MacroTrap (6	TR125109/58	$395.00
ISRP Protein Removal MicroTrap	TR1/25108/08	$ 75.00
ISRP Protein Removal MicroTrap (6	TR1/25109/08	$395.00

Holders:

Holder Description	Part Number	Price
Manual MacroTrap Holder Kit	TH1/25111/02	$125.00
Manual MicroTrap Holder Kit	TH1/25111/01	$125.00
Manual CapTrap Holder Kit	TH1/25029/03	$125.00

Holder Description	Part Number	Price
Cheminert CapTrap Loop/Holder	TH1/25029/05	$125.00
Cheminert Micro/MacroTrap Loop/Holder	TH1/25110/02	$125.00
Valco CapTrap Loop/Holder	TH1/25029/00	$125.00
Valco Micro/MacroTrap Loop/Holder	TH1/25110/00	$125.00
Rheodyne CapTrap Loop/Holder	TH1/25029/01	$125.00
Rheodyne Micro/MacroTrap Loop/Holder	TH1/25111/00	$125.00

Part Numbers & Pricing Information

Traps:

Trap Description	Part Number	Price
Small Molecule MacroTrap (Each)	TR1/25108/51	$ 75.00
Small Molecule MacroTrap (6 Pack)	TR1/25109/51	$395.00
Small Molecule MicroTrap (Each)	TR1/25108/01	$ 75.00
Small Molecule MicroTrap (6 Pack)	TR1/25109/01	$395.00
Small Molecule CapTrap (Each)	TR1/25108/31	$ 75.00
Small Molecule CapTrap (6 Pack)	TR1/25109/31	$395.00

Trap Description	Part Number	Price
Peptide MacroTrap (Each)	TR1/25108/52	$ 75.00
Peptide MacroTrap (6 Pack)	TR1/25109/52	$395.00
Peptide MicroTrap (Each)	TR1/25108/02	$ 75.00
Peptide MicroTrap (6 Pack)	TR1/25109/02	$395.00
Peptide CapTrap (Each)	TR1/25108/32	$ 75.00
Peptide CapTrap (6 Pack)	TR1/25109/32	$395.00

Trap Description	Part Number	Price
Protein MacroTrap (Each)	TR1/25108/53	$ 75.00
Protein MacroTrap (6 Pack)	TR1/25109/53	$395.00
Protein MicroTrap (Each)	TR1/25108/03	$ 75.00
Protein MicroTrap (6 Pack)	TR1/25109/03	$395.00
Protein CapTrap (Each)	TR1/25108/33	$ 75.00
Protein CapTrap (6 Pack)	TR125109/33	$395.00

Trap Description	Part Number	Price
SCX MacroTrap (Each)	TR1/25108/55	$ 75.00
SCX MacroTrap (6 Pack)	TR1/25109/55	$395.00
SCX MicroTrap (Each)	TR1/25108/05	$ 75.00
SCX MicroTrap (6 Pack)	TR1/25109/05	$395.00
SCX CapTrap (Each)	TR1/25108/35	$ 75.00
SCX CapTrap (6 Pack)	TR1/25109/35	$395.00

General Tips for Michrom Trap Cartridges

Installation into the holder

1. Dry the outside surface of the trap cartridge
2. Dry the internal surface of the trap holder
3. Place the trap inside the holder
   (Note: the traps are bi-directional)
4. Tighten the holder "finger tight"
5. Tighten the holder $^{1}/_{8}$ to $^{1}/_{4}$ of a turn with a pair of wrenchs

Use

Refer to details outlined in this manual for each specific type of trap cartridge utilized.

Storage

Flush with 40/40/20 Acetonitrile/n-Propanol/H_2O
or a similar 50/50 Alcohol/H_2O Solution.

Cleaning & Regeneration

Refer to details outlined in this brochure for each specific type of trap cartridge utilized.

Trouble-shooting

Follow the detailed instructions provided within this manual for successful trapping and/or detergent removal.

If low recoveries are noted with a new trap, re-equilibrate the trap and perform a second run. If the low recoveries are still apparent, re-install the trap in its holder to rule-out any leak age. Re-equilibrate the trap and perform another run. If low recoveries persist, call Michrom for technical assistance.

Through time, a reduction in trapping efficiency may be noted. Follow the cleaning & regeneration procedure outlined in this manual prior to installing a new trap.

Small Molecule Concentration & Desalting Trap

♦ Band Color = Purple

♦ Contains a small pore, large particle, hydrophilic C18 silica (ODS-AQ) reversed-phase packing material

♦ Designed to bind small molecules (0.1-10 kD) including pharmaceuticals, petrochemicals, natural products, and other organic molecules

♦ Concentrates samples up to 100 fold

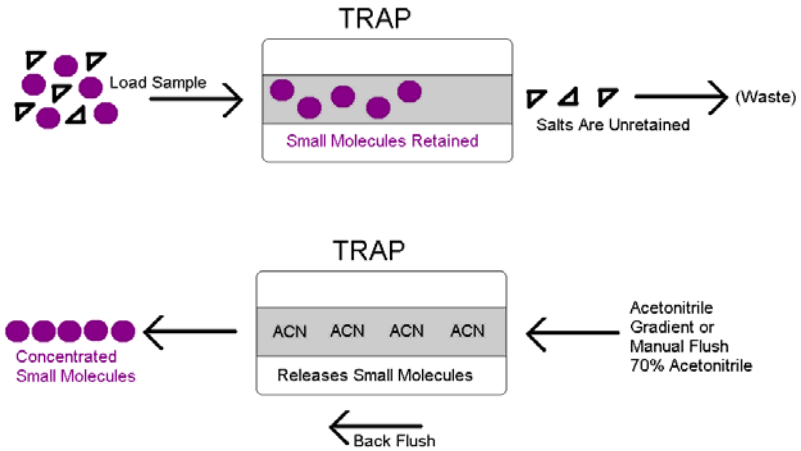
♦ Removes salts (8M) and non-volatile buffers

♦ pH range 2-7.5

♦ Clean and/or Regenerate using IPA

Cartridge Type	Internal Dimensions	Bed Volume	Sample Capacity	Sample Volume	Speed of Loading*
MacroTrap™	3 x 8 mm	50 µl	200 µg	10-10000 µl	500-2000 µl/min
MicroTrap™	1 x 8 mm	5.0 µl	20 µg	1.0-1000 µl	50-200 µl/min
CapTrap™	0.5 x 2 mm	0.5 µl	2 µg	0.1-100 µl	5-20 µl/min

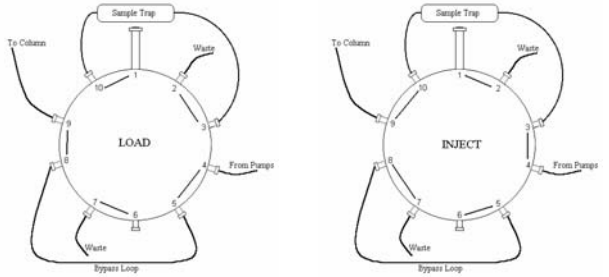
**Note: Use this speed of loading range for optimal recoveries*



ISRP Protein Removal Trap

(Continued)

Example Plumbing for On-line Trapping



ManualTrapping

Instructions for Successful Trapping

1. ***Clean the trap*** with 5-10 trap volumes of "B Solvent" (typically 90/10/0.005-0.1% Acetonitrile/H_2O/Ion-Pairing acid such as TFA or HFBA). *Note: HFBA = Heptafluorobutyric Acid*

2. ***Equilibrate the trap*** with 5-10 trap volumes of Equilibration Buffer, pH 7.0
   Example Buffer: 5/95 Acetonitrile/180mM Ammonium Acetate
   Or 5/95 Acetonitrile/0.5M Diisopropylethylamine (DIEA-titrated with acetic acid to pH 7.0).
   Note: Some Small Molecules may require 2% ACN rather than 5%

3. ***Add appropriate amount of Acetonitrile and Buffer to sample*** to equal Equilibration Buffer.

4. ***Load sample*** onto trap at a loading rate within the recommended speed of loading for the size of trap in use. Do not overload the trap.

5. ***Remove proteins and salts*** from trap and flush to waste by washing with approximately 5 trap volumes of Equilibration Buffer.

6. ***Elute small molecules*** from trap. If performing on-line trapping, toggle the valve to the Inject position and then run an increasing gradient of Acetonitrile. For manual trapping, flush the trap with 1-2 trap volumes of 65-90% Acetonitrile or "B Solvent".

ISRP Protein Removal Trap

♦ Band Color = Yellow

♦ Contains a very small pore, large particle, silica-based internal surface, reversed-phase packing material

♦ Designed to bind small molecules (0.1-5 kD) including pharma-ceuticals onto the C18 chains within the internal surface of the pores of the packing material

♦ Concentrates samples up to 100 fold

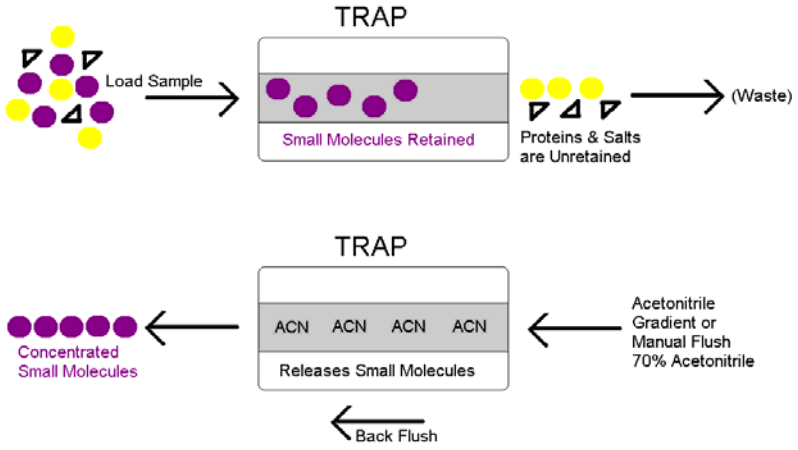
♦ Removes proteins from plasma, urine and serum samples by excluding the proteins from the shielded hydrophobic phase and allowing them to pass through the inter-particulate spaces

♦ pH range 2-7.5

♦ Clean and/or Regenerate using IPA

Cartridge Type	Internal Dimensions	Bed Volume	Sample Capacity	Sample Volume	Speed of Loading*
MacroTrap™	3 x 8 mm	50 µl	200 µg	10-10000 µl	500-2000 µl/min
MicroTrap™	1 x 8 mm	5.0 µl	20 µg	1.0-1000 µl	50-200 µl/min
CapTrap™	0.5 x 2 mm	0.5 µl	2 µg	0.1-100 µl	5-20 µl/min

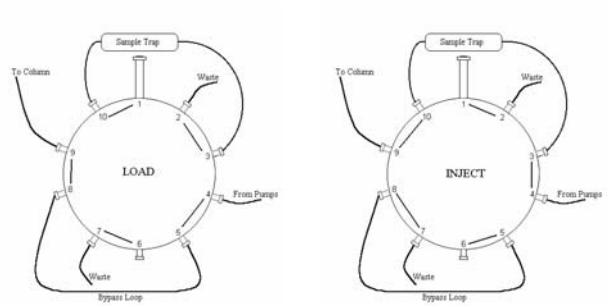
**Note: Use this speed of loading range for optimal recoveries*



Small Molecule Concentration & Desalting Trap

(Continued)

Example Plumbing for On-line Trapping



ManualTrapping

Instructions for Successful Trapping

1. ***Clean the trap*** with 5-10 trap volumes of "B Solvent" (typically 90/10/0.005-0.1% Acetonitrile/H_2O/Ion-Pairing Acid such as TFA or HFBA). *Note: HFBA = Heptafluorobutyric Acid*

2. ***Equilibrate the trap*** with 5-10 trap volumes of "A Solvent" (typically 2/98/0.005-0.1% Acetonitrile/H_2O/Ion-Pairing Acid such as TFA or HFBA).

3. ***Add appropriate amount of Acetonitrile and Ion-Pairing acid to sample*** to equal "A Solvent".

4. ***Load sample*** onto trap at a loading rate within the recommended speed of loading for the size of trap in use. Do not overload the trap.

5. ***Remove salts*** from trap and flush to waste by washing with approximately 5 trap volumes using "A Solvent".

6. ***Elute small molecules*** from trap. If performing on-line trapping, toggle the valve to the Inject position and then run an increasing gradient of Acetonitrile. For manual trapping, flush the trap with 1-2 trap volumes of 65-90% Acetonitrile or "B Solvent".

Peptide Concentration & Desalting Trap

♦ Band Color = Green

♦ Contains a medium pore, large particle, polymeric reversed-phase packing material with retention similar to a C8 phase

♦ Designed to bind protein digests, peptides, small poly-neucleotides and other small biological molecules (0.5-50kD)

♦ Concentrates samples up to 100 fold

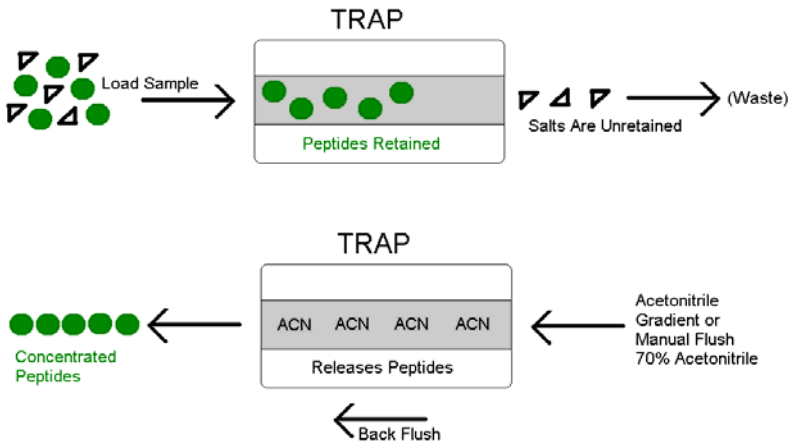
♦ Removes salts (8M) and nonvolatile buffers

♦ pH range 1-13

♦ Clean and/or Regenerate using 70:30 conc. Formic Acid:IPA

Cartridge Type	Internal Dimensions	Bed Volume	Sample Capacity	Sample Volume	Speed of Loading*
MacroTrap™	3 x 8 mm	50 µl	200 µg	10-10000 µl	500-2000 µl/min
MicroTrap™	1 x 8 mm	5.0 µl	20 µg	1.0-1000 µl	50-200 µl/min
CapTrap™	0.5 x 2 mm	0.5 µl	2 µg	0.1-100 µl	5-20 µl/min

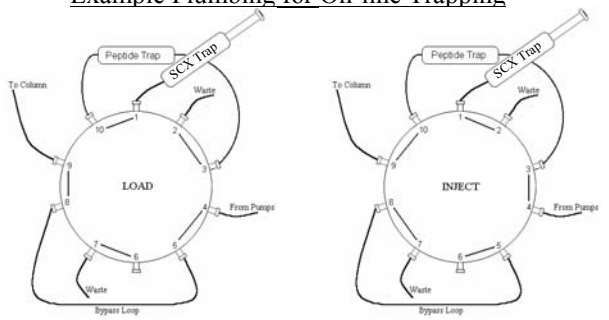
**Note: Use this speed of loading range for optimal recoveries*



SCX (Strong Cation Exchange) Trap

(Continued)

Example Plumbing for On-line Trapping



ManualTrapping

Instructions for Successful Trapping

1. ***Clean the trap*** with 5-10 trap volumes of "High Salt Buffer, pH 3" of choice. *Examples: 5mM NaH_2PO_4 , pH 3.0, with 25% Acetonitrile + 0.25M KCl. Note: If using a Peptide Conc. & Desalting Trap in tandem with SCX Trap for 2D Analysis, a good buffer is 5/90/2.5/2.5/0.05% Acetonitrile/H_2O/30% Ammonium Hydroxide/ Formic Acid/HFBA ("D" Buffer)*

2. ***Equilibrate the trap*** with 5-10 trap volumes of "Low Salt Buffer". *Example: 5mM NaH_2PO_4 , pH 3.0, with 25% Acetonitrile Note: If using a Peptide Conc. & Desalting Trap in tandem with SCX Trap for 2D Analysis, a good buffer is 5/95/0.1/0.005% Acetonitrile/H_2O/Formic Acid/HFBA ("C" Buffer)*

3. ***Add appropriate amount of Acetonitrile and Buffer to the Sample*** to obtain pH 3.0 & 25% Acetonitrile. *Note: Match"C" Buffer for 2D.*

4. ***Load sample*** onto trap at a loading rate within the recommended speed of loading for the size of trap in use. Do not overload the trap.

5. ***Release peptides from the Trap*** using 1-2 Trap Volumes of "High Salt Buffer" or perform salt steps with increasing concentrations of salt. If performing on-line trapping, then load 5-10 trap volumes of "C Buffer" to allow concentration & desalting of peptides on Pept. Trap

6. ***Toggle valve to Inject & Elute peptides from concentration & desalting trap*** by running an increasing gradient of Acetonitrile.

SCX (Strong Cation Exchange) Trap

♦ Band Color = Orange

♦ Contains a medium pore, large particle, silica-based strong cation exchange material (PolySulfoethyl Aspartamide)

♦ Designed to bind protein digests, peptides, and other small (+) charged molecules (0.5-50kD) for 1D or 2D analysis

♦ Peptides lose their (-) charge , and have a net (+) charge at pH 2.7-3.0

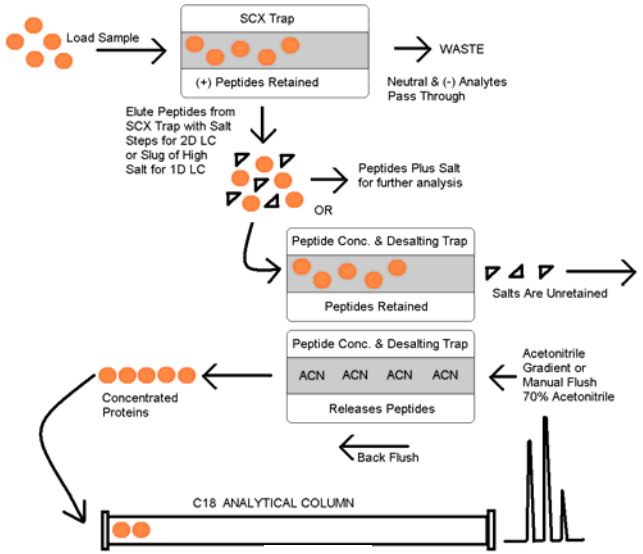
♦ Concentrates samples up to 100 fold

♦ pH range 2.7-7.0 **Note: pH less than 2.7 will destroy phase**

♦ Clean +/or Regenerate using High Salt Buffer of Choice

Cartridge Type	Internal Dimensions	Bed Volume	Sample Capacity	Sample Volume	Speed of Loading*
MacroTrap™	3 x 8 mm	50 µl	800 µg	10-10000 µl	500-2000 µl/min
MicroTrap™	1 x 8 mm	5.0 µl	80 µg	1.0-1000 µl	50-200 µl/min
CapTrap™	0.5 x 2 mm	0.5 µl	8 µg	0.1-100 µl	5-20 µl/min

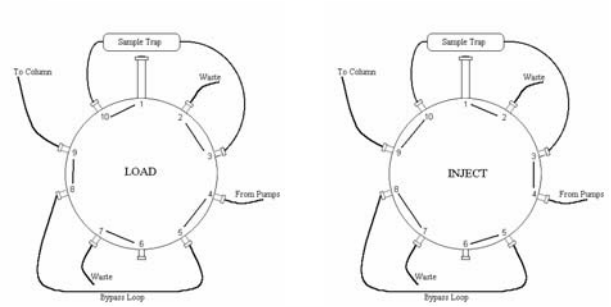
**Note: Use this speed of loading range for optimal recoveries*



Peptide Concentration & Desalting Trap

(Continued)

Example Plumbing for On-line Trapping



ManualTrapping

Instructions for Successful Trapping

1. ***Clean the trap*** with 5-10 trap volumes of "B Solvent" (typically 90/10/0.005-0.1% Acetonitrile/H_2O/Ion-Pairing Acid such as TFA or HFBA). *Note: HFBA = Heptafluorobutyric Acid*

2. ***Equilibrate the trap*** with 5-10 trap volumes of "A Solvent" (typically 2/98/0.005-0.1% Acetonitrile/H_2O/Ion-Pairing Acid such as TFA or HFBA).

3. ***Add appropriate amount of Acetonitrile and Ion-Pairing acid to sample*** to equal "A Solvent".

4. ***Load sample*** onto trap at a loading rate within the recommended speed of loading for the size of trap in use. Do not overload the trap.

5. ***Remove salts*** from trap and flush to waste by washing with approximately 5 trap volumes using "A Solvent".

6. ***Elute peptides*** from trap. If performing on-line trapping, toggle the valve to the Inject position and then run an increasing gradient of Aceto-nitrile. For manual trapping, flush the trap with 1-2 trap volumes of 65-90% Acetonitrile or "B Solvent".

Protein Concentration & Desalting Trap

♦ Band Color = Black

♦ Contains a large pore, large particle, polymeric reversed-phase packing material with retention similar to a C4 phase

♦ Designed to bind proteins, polyneucleotides and other large biological molecules (5-500kD)

♦ Concentrates samples up to 100 fold

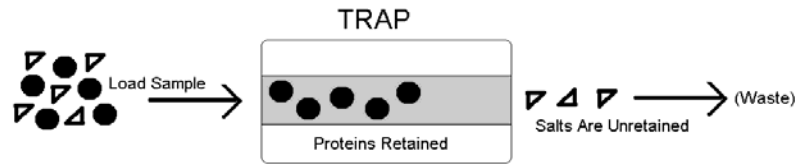
♦ Removes salts (8M) and nonvolatile buffers

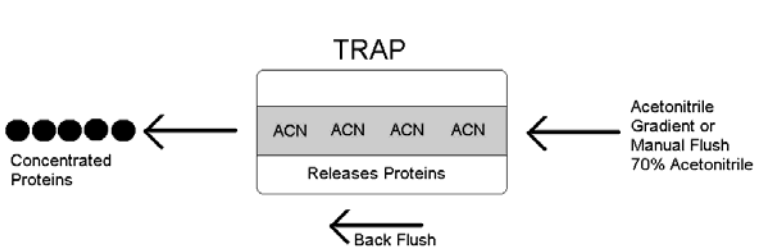
♦ pH range 1-13

♦ Clean and/or Regenerate using 70:30 Formic Acid:IPA

Cartridge Type	Internal Dimensions	Bed Volume	Sample Capacity	Sample Volume	Speed of Loading*
MacroTrap™	3 x 8 mm	50 µl	200 µg	10-10000 µl	500-2000 µl/min
MicroTrap™	1 x 8 mm	5.0 µl	20 µg	1.0-1000 µl	50-200 µl/min
CapTrap™	0.5 x 2 mm	0.5 µl	2 µg	0.1-100 µl	5-20 µl/min

**Note: Use this speed of loading range for optimal recoveries*

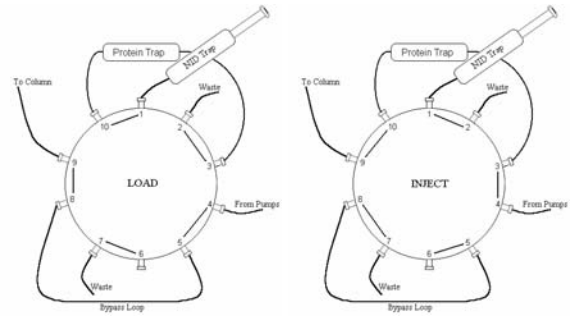




NID (Non-Ionic Detergent) Removal Trap

(Continued)

Example Plumbing for On-line Trapping



ManualTrapping

Instructions for Successful Trapping

1. ***Clean the trap*** with 5-10 trap volumes of 10% Acetonitrile/0.5M NaCl.

2. ***Equilibrate the trap*** with 5-10 trap volumes of 10% Acetonitrile/10mM Buffer*, pH 7.0 (or some other pH not corresponding to the pI of proteins). **Examples: Ammonium Acetate Buffer, Phosphate Buffer, or DIEAAc (di-Isopropalethylamine Acetate) Buffer*

3. ***Add appropriate amount of Acetonitrile and Buffer Solution to sample*** to allow sample to contain 10% Acetonitrile buffered at pH 7.0 (or at some other pH not corresponding to the pI of proteins).

4. ***Load sample*** onto trap at a loading rate within the recommended speed of loading for the size of trap in use. Do not overload the trap. NID will pass through trap while proteins remain on NID Removal Trap.

5. ***Release proteins from the NID Removal Trap*** using 1-2 Trap Volumes of 10% ACN/0.5M NaCl. If performing on-line trapping, then load 5-10 trap volumes of "A Solvent" (typically 2/98/0.005-0.1% ACN/H_2O/Ion-Pairing Acid like TFA or HFBA) to allow concentration & desalting of proteins on the Protein Trap (refer to plumbing diagram). *Note: Some proteins require upto 5% ACN in "A Solvent"*

6. ***Toggle valve to Inject & Elute proteins*** from concentration & desalting trap by running an increasing gradient of Acetonitrile.

NID (Non-Ionic Detergent) Removal Trap

♦ Band Color = Blue

♦ Contains a mixed bed of large pore, large particle, silica based weak anion and weak cation exchange packing material

♦ Designed to bind charged proteins and/or peptides

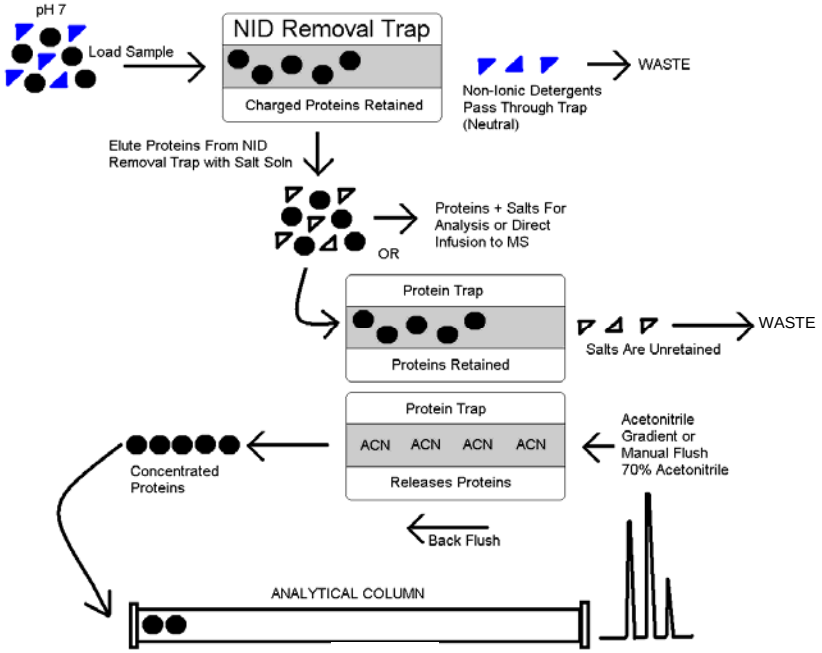
♦ Removes Non-Ionic Detergents such as Triton X-100 and Tween-80 by allowing the neutral detergents to pass through

♦ pH range 2-7.5

♦ Clean +/or Regenerate using 10% Acetonitrile/0.5M NaCl

Cartridge Type	Internal Dimensions	Bed Volume	Sample Capacity	Sample Volume	Speed of Loading*
MacroTrap™	3 x 8 mm	50 µl	200 µg	10-10000 µl	500-2000 µl/min
MicroTrap™	1 x 8 mm	5.0 µl	20 µg	1.0-1000 µl	50-200 µl/min
CapTrap™	0.5 x 2 mm	0.5 µl	2 µg	0.1-100 µl	5-20 µl/min

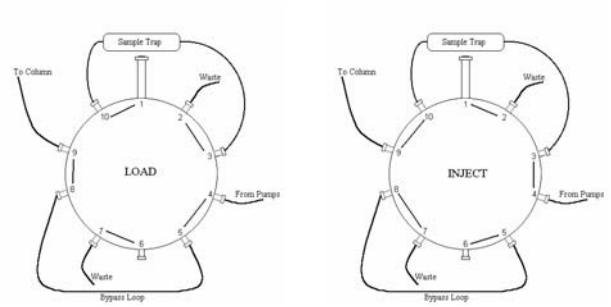
**Note: Use this speed of loading range for optimal recoveries*



Protein Concentration & Desalting Trap

(Continued)

Example Plumbing for On-line Trapping



ManualTrapping

Instructions for Successful Trapping

1. ***Clean the trap*** with 5-10 trap volumes of "B Solvent" (typically 90/10/0.005-0.1% Acetonitrile/H_2O/Ion-Pairing Acid such as TFA or HFBA). *Note: HFBA = Heptafluorobutyric Acid*

2. ***Equilibrate the trap*** with 5-10 trap volumes of "A Solvent" (typically 2/98/0.005-0.1% Acetonitrile/H_2O/Ion-Pairing Acid such as TFA or HFBA). *Note: Some proteins require up to 5% ACN in "A Solvent"*

3. ***Add appropriate amount of Acetonitrile and Ion-Pairing acid to sample*** to equal "A Solvent".

4. ***Load sample*** onto trap at a loading rate within the recommended speed of loading for the size of trap in use. Do not overload the trap.

5. ***Remove salts*** from trap and flush to waste by washing the trap with approximately 5 trap volumes using "A Solvent".

6. ***Elute proteins*** from trap. If performing on-line trapping, toggle the valve to the Inject position and then run an increasing gradient of Aceto-nitrile. For manual trapping, flush the trap with 1-2 trap volumes of 65-90% Acetonitrile or "B Solvent".

SDS Removal Trap

♦ Band Color = Red

♦ Contains a large pore, large particle, polymeric strong anion exchange packing material

♦ Designed to bind Sodium dodecyl sulfate (SDS) and other anionic detergents at low pH (2-4)

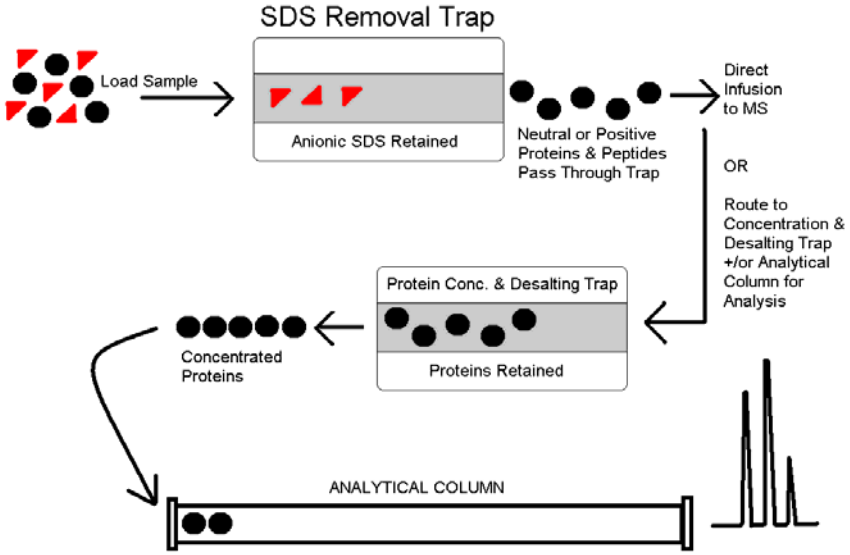
♦ Removes concentrations as high as 1% SDS.
Note: Higher concentrations of SDS form micelles and trap the analytes along with the SDS micelle complex (these samples must be diluted below 1% prior to analysis)

♦ pH range 1-13

♦ Clean and/or Regenerate using 90% Acetonitrile/0.1% HCl

Cartridge Type	Internal Dimensions	Bed Volume	SDS Removal Limits	Speed of Loading*
MacroTrap™	3 x 8 mm	50 µl	1.0 mg (100µL of 1% SDS)	500-2000 µl/min
MicroTrap™	1 x 8 mm	5.0 µl	0.1 mg (10µL of 1% SDS)	50-200 µl/min

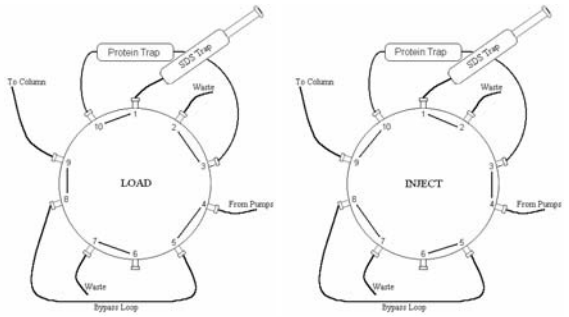
**Note: Use this speed of loading range for optimal recoveries*



SDS Removal Trap

(Continued)

Example Plumbing for On-line Trapping



ManualTrapping

Instructions for Successful Trapping

1. ***Clean the trap*** with 5-10 trap volumes of "B Solvent" (typically 90/10/0.005-0.1% Acetonitrile/H_2O/Ion-Pairing Acid such as TFA or HFBA). *Note: HFBA = Heptafluorobutyric Acid*

2. ***Equilibrate the trap*** with 5-10 trap volumes of "A Solvent" (typically 2/98/0.005-0.1% Acetonitrile/H_2O/Ion-Pairing Acid such as TFA or HFBA). *Note: Some proteins require up to 5% ACN in "A Solvent"*

3. ***Add appropriate amount of Acetonitrile and Ion-Pairing acid to sample*** to equal "A Solvent". *Note: pH must be 2-4*

4. ***Load sample*** onto trap at a loading rate within the recommended speed of loading for the size of trap in use. Do not overload the trap. SDS will bind to trap while proteins pass through.

5. ***Capture proteins*** as they pass through SDS Removal Trap for further analysis. If performing on-line trapping, add 5-10 trap volumes of "A Solvent" to allow concentration & desalting of proteins on the Protein Trap (refer to plumbing diagram). If performing manual SDS Removal, add 1-2 trap volumes of "A Solvent" to allow all proteins to pass through SDS Removal Trap.

6. ***Toggle valve to Inject & Elute proteins*** from concentration & desalting trap by running an increasing gradient of Acetonitrile. While in Inject Position, also ***clean the SDS Trap & route retained SDS to waste*** by flushing with 5-10 trap volumes of 90% ACN/0.1% HCl.