Solvents

One Call. One Source.
A World of Solvents and Reagents.

- Reliable for GC Derivatization
- Pure for HPLC Application
- Reproducible for Immunohistochemistry

MP

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MP Biomedicals Solvent Grade Selection Guide

Type/Grade	Explanation	Application
ACS	Reagent chemicals that meet or exceed the latest ACS Specifications. Actual lot analysis on label.	Analytical applications requiring high purity specifications
Anhydrous	Solvents that contain very low water content, typically less than 50 ppm.	Organic, Organometallic, Oligonucleotide Synthesis, and for moisture sensitive reactions
Certified	Solvents whose purity is guaranteed to meet published maximum limits of impurities.	General analytical applications
GC	Solvents with the highest purity and lot-to-lot consistency for Gas Chromatography (GC). Free of contaminants to ppb level.	Gas Chromatography
Histological	Solvents specifically prepaid or used in the Histology laboratory setting and are filtered for Tissue Processing applications.	Tissue processing
HPLC	Solvents suitable for use with High Performance Liquid Chromatography (HPLC) instruments, Ultraviolet (UV) Spectrophotometric needs and general laboratory use. Submicron filtered and controlled for high assay and low UV absorption, fluorescence, residue, and water.	HPLC and Spectrophotometry procedures
HPLC/Spectro	High purity solvents made for use with HPLC instruments and UV Spectrophotometric procedures.	HPLC and Spectrophotometry procedures
Low Benzene	Solvents that contain very low benzene content.	Hydrocarbon analysis and Spectrophotometric applications
Molecular Biology	Solvents that are suitable for molecular biological applications such as PCR, NGS and Gel Electrophoresis.	General laboratory and Molecular Biology applications
Spectro	Solvents for use in Spectrophotometry.	Spectrophotometry procedures
Ultra Pure	Solvents for applications where high assay and lot-to-lot consistency are critical.	HPLC, Liquid Chromatography (LC), GC, Spectrophotometry and general laboratory use.
USP/NF	Solvents that meet or exceed specifications of United States Pharmacopoeia and National Formulary.	Food, Drug and Cosmetic applications.

MP Biomedicals High Purity Solvents Selection Guide

Solvent	UV Cutoff (nm)	Boiling Point (°C)	Density (g/mL, 25°C)	Refractive Index (25°C)
Acetone	330	56.1	0.7857	1.3568
Acetonitrile	190	81.6	0.778	1.3415
1-Butanol	215	117.7	0.8098	1.3972
Chloroform	245	61.7	1.484	1.4445
Cyclohexane	202	80.7	0.774	1.4247
N,N-Dimethylformamide	268	153	0.944	1.428
Dimethyl Sulfoxide	262	189	1.1014	1.4783
EthylAcetate	255	77.1	0.894	1.3695
Ethyl Ether	218	34.6	0.7134	1.35
Glycerol	205	290	1.2613	1.4746
Heptane	197	98.4	0.6838	1.3855
Hexane	195	69	0.663	1.3759
Isooctane	205	99.2	0.6919	1.3895
Methanol	205	64.7	0.7915	1.3288
MethyleneChloride	233	39.5	1.318	1.4215
N-Methylpyrrolidinone	275	202.2	1.03	1.469
Pentane	190	36.1	0.6264	1.3555
Petroleum Ether	-	35-60	0.64	1.361
2-Propanol	205	82.3	0.7855	1.3772
Tetrahydrofuran	210	66.1	0.8892	1.406
Toluene	285	110.6	0.866	1.494
Water	-	100	0.9982	1.333

Melting Point (°C)	Polarity Index (P')	Eluotropic Value on Silica (D°)	Viscosity (cP, 20°C)	Flash Point (°C)	Mol. Wt.
-94.3	5.1	0.53	0.36	20	58.08
-50	5.8	0.52	0.36	2	41.05
-88.6	3.9	-	2.98	35	74.12
-63.3	4.1	0.26	0.58	N/A	119.38
-6.5	0.2	0.03	0.9	-20	84.16
-61	6.4	-	0.92	58	73.09
18.5	7.2	-	2.24	87.8	78.13
-83.9	4.4	0.38	0.45	-4	88.11
-116.3	2.8	0.43	0.24	-45	74.12
18.2	-	-	1413.8	193	92.09
-90.6	0.2	0.01	0.4	-4	100.2
-95.3	0.1	0.01	0.31	-23	86.18
109.5	0.1	0.01	0.5	28	114.23
-97.8	5.1	0.73	0.55	12	32.04
-96.7	3.1	0.32	0.3	N/A	84.93
-24.4	-	-	1.67	95	99.13
-129.7	0	0	0.22	-49	72.15
-	0.1	-	-	-18	-
-90	3.9	0.63	2.4	-12	60.1
-108.3	4	0.35	0.55	-14	72.11
-95	2.4	0.22	0.59	-4	92.14
0	10.2	-	1	N/A	18.02

Derivatization Reagents and Solvents for Gas Chromatography

Chemical derivatization is used to convert the non-UV absorbing analytes into forms that are easily chromatographed or detected with high sensitivity. GC derivatization is frequently used to simplify complex separation problems.

Samples containing functional groups with active hydrogen atoms (-COOH, -OH, -NH and -SH) are often difficult to analyze by GC because they are not sufficiently volatile, show excessive tailing, can be too strongly attracted to the stationary phase or are thermally unstable.

The majority of derivatization reactions commonly used for gas chromatography applications are categorized into three types: Silylation, Acylation and Alkylation & Esterification.

GC samples are derivatized prior to analysis to:

- Increase the volatility and decrease the polarity of the compound
- Reduce thermal degradation of samples by increasing their thermal stability
- Increase detector response by incorporating functional groups which lead to higher detector signals, e.g. CF_3 groups for electron capture
- Improve separation and reduce tailing
- Enlarge substrate spectrum

Silylation Reagents

Silyl derivatives are the most widely used derivatives for gas chromatographic applications. The silyl reagents have two desirable results: increase analyte volatility and decrease surface adsorption. Usually they are formed by the replacement of the active hydrogens from acids, alcohols, thiols, amines, amides and enolizable ketones and aldehydes with the trimethylsilyl group. A typical reaction is shown below.

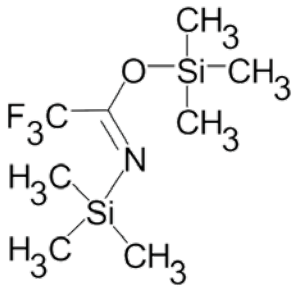
$$\text{Sample-OH} + R_3Si \longrightarrow X\ \text{Sample-O-Si-}R_3 + HX$$

It involves nucleophilic attack upon the silicon atom of the silyl donor, producing a bimolecular transition state. The ideal silyl compound leaving group (X) must be such that it is readily lost from the transition state during the reaction, but possesses sufficient chemical stability in combination with the alkyl silyl group to allow long term storage of the derivatizing agent for use as required.

BSTFA – N,O-Bis(trimethylsilyl)trifluoroacetamide

Cat. No. 02150476 | 5 g, 25 g

The greatest advantage of using MP Bio BSTFA over other silylating reagents is the increased volatility of its byproducts, mono(trimethylsilyl) trifluoroacetamide and trifluoroacetamide. This increased volatility results in the byproducts eluting with the solvent front, providing excellent chromatographic separations.



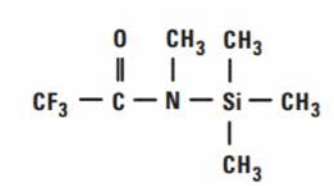
Molecular Formula	$CF_3C=NSi(CH_3)_3OSi(CH_3)_3$
CAS Number	25561-30-2
Formula Weight	257.40
Bp	40°C / 12 mm
Flash Point	23°C
d	0.96
n_D	1.384 at 20°C
Appearance	Clear, colorless to very light yellow liquid, moisture sensitive

Protocol:

1. Combine 5-10 mg sample, 0.5 mL BSTFA and 1.0 mL solvent (acetonitrile is recommended for amino acids) in a 3.0 mL small reaction vial.
2. Cap vial and shake for 30 seconds.
3. Heat at 70°C for 15 minutes.
4. Analyze by gas chromatography.

MSTFA – N-Trimethylsilyl-N-methyltrifluoroacetamide

Cat. No. 02155675 | 5 g, 25 g



- Trimethylsilyl donor strength comparable to BSA and BSTFA
- Reacts to replace labile hydrogens on a wide range of polar compounds with a $-Si(CH_3)_3$ group
- Used to prepare volatile and thermally stable derivatives for GC and GC/MS
- Primary advantage of MP Bio MSTFA is the volatility of its byproduct, N-methyltrifluoroacetamide; MSTFA is the most volatile TMS-amide available which has an even lower retention time than MSTFA
- Often TMS derivatives of small molecules can be analyzed when derivatized with MSTFA because the byproducts and reagent itself usually elute with the solvent front
- Addition of TMCS aids derivatization of amides, secondary amines and hindered hydroxyls not derivatized by MSTFA alone

Molecular Formula	$CF_3CON(CH_3)Si(CH_3)_3$
CAS Number	24589-78-4
Formula Weight	199.25
Bp	130-132°C
n_D	1.38 at 20°C
Appearance	Clear, colorless to pale yellow liquid

Protocol:

1. Combine 5-10 mg sample, 0.5 mL MSTFA and 1.0 mL solvent (acetonitrile is recommended for amino acids) in a 3.0 mL small reaction vial.
2. Cap vial and shake for 30 seconds.
3. Heat at 70°C for 15 minutes.
4. Analyze by gas chromatography

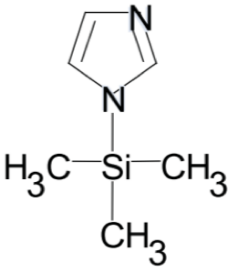
Derivatization Reagents and Solvents for Gas Chromatography

TMSI – N-Trimethylsilylimidazole

Cat. No. 02152172 | 25 mL, 100 mL

Protocol:

1. Weigh 1-10 mg of sample into 5 mL reaction vessel. If required, dissolve in solvent.
2. Add excess silylating reagent (minimum a 2:1 molar ratio of TMSI to active hydrogen).
3. Analyze aliquots of the sample at selected time intervals until no further increase in product peak(s) is observed, which indicates that the reaction is complete.



Molecular Formula	$(CH_3)_3SiNCH=NCH=CH$
CAS Number	18156-74-6
Formula Weight	140.26
Bp	93-94°C / 14 mm
Flash Point	5°C
d	0.956
n_D	1.470 at 20°C
Appearance	Clear, colorless to very light yellow liquid, moisture sensitive

Other Silylation Reagents for GC Derivatization

Description	CAS Number	Cat. No.
HMDSO, Hexamethyldisiloxane	107-46-0	0215124980

Silylation Grade Solvents

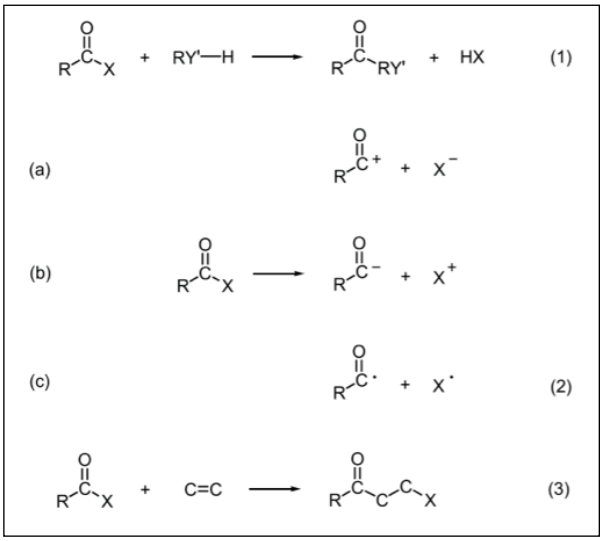
MP Bio Silylation Grade Solvents are specially manufactured and packaged to meet the exacting needs of silylation and other sensitive derivatization reactions.

Description	Size	Cat. No.
Acetonitrile, HPLC/Spectro Grade, ≥99.9%	4 L	02300008
	4 x 4 L	
Dimethyl sulfoxide > 99%	1 L	02190186
	100 mL	
	500 mL	
Pyridine, Triple Distilled, ACS Grade	100 mL	02151983
	250 mL	
Tetrahydrofuran	4 x 4 L	02300200
	6 x 1 L	

Acylation

Acylation, an alternative to silylation, is the conversion of compounds with active hydrogen such as -OH, -SH, and -NH into esters, thioesters and amides. In addition, alkylation reactions can be used to prepare ethers, thioethers and thioesters; N-alkylamines; and amides. The general reaction for acylation is shown in the equations to the right.

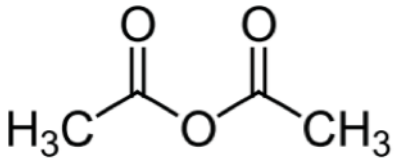
Acylation involves the introduction of an acyl group into a molecule with a replaceable hydrogen atom. In the above equation R-C(=O)-X is the acylation reagent and R'Y-H is the target analyte, equation (1). The acylating agent R-C(=O)-X can lose the group –X by (a) electrophilic, (b) nucleophilic or (c) free radical mechanisms as represented in equation (2). Less commonly, an acyl group may be added across a double bond, equation (3).



Acetic Anhydride

Cat. No. 02154680 | 500 mL, 1000 mL

- Acylation is an alternative to silylation, producing stable, volatile derivatives of alcohols, phenols, and amines for analysis by GC/FID.
- Acylated compounds are more stable than corresponding silylated compounds.



Molecular Formula	$(CH_3CO)_2O$
CAS Number	108-24-7
Formula Weight	102.09
d	1.080-1.085
n_D	1.3901
Appearance	Clear, colorless liquid

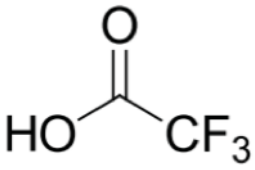
Protocol:

1. Dissolve 5 mg of sample in 5 mL of chloroform.
2. Add 0.5 mL acetic anhydride and 1 mL of acetic acid. Heat at 50°C for 2-16 hours.
3. Remove excess reagent by evaporating the mixture to dryness and redissolve the residue in chloroform for GC analysis

Derivatization Reagents and Solvents for Gas Chromatography

TFA – Trifluoroacetic Acid

Cat. No. 02300214 | 500 mL, 6 x 500 mL



- Silyl Catalyst - Addition of a small amount of acidic catalyst usually increases the rate or degree of silylation. In acid catalysis, protonation of the silyl donor weakens the Si-X bond (X is the leaving group).
- TFA derivatives are stable and volatile. Use of TFA in combination with HMDS avoids the formation of ammonium chloride.

Molecular Formula	CF_3COOH
CAS Number	76-05-1
Formula Weight	114.02
Bp	72.4°C
d	1.480
Appearance	Clear, colorless liquid, moisture sensitive

Protocol:

Carbohydrates in syrups

1. Place 60-70 mg of 80% soluble syrup in a vial and dissolve with pyridine.
2. Add 900 µL HMDS, then 100 µL TFA. Shake 30 seconds, then let stand 15 minutes with occasional shaking. Inject aliquot on to GC column for analysis.
3. To determine when derivatization is complete, analyze aliquots of the sample at selected time intervals until no further increase in product peak(s) is observed, indicating reaction completion.

Aflatoxins

1. Place extracted sample containing aflatoxins in screw cap vial. Evaporate to dryness using clean nitrogen.
2. Add 200 µL of hexane to re-dissolve aflatoxins.
3. Add 50 µL of TFA, cap, and vortex for 30 sec. Let stand for 5 min.
4. Add 2.0 mL deionized water:acetonitrile (9:1). Vortex for 30 seconds, then allow layers to separate.
5. Remove aqueous layer containing aflatoxins. Filter through 0.45 µm syringe-tip filter and inject aliquot into LC column.

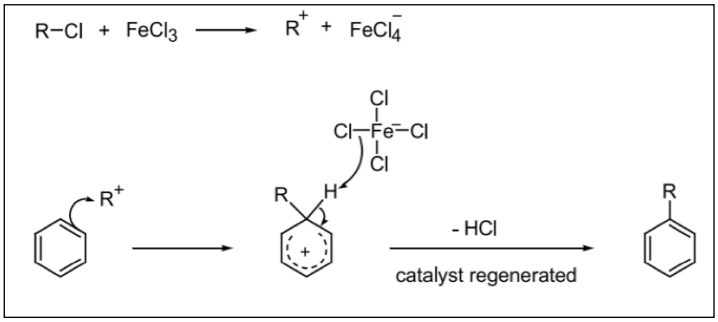
Other Acylation Reagents for GC Derivatization

Description	CAS Number	Cat. No.
Benzoic Acid	65-85-0	02191412
Ethyl Acetoacetate	141-97-9	05213382
1-Acetylimidazole	2466-76-4	02150231
Hexafluoroacetylacetone	1522-22-1	02157354
2,3,4,5,6-Pentafluorobenzaldehyde	653-37-2	05221367

Alkylation and Alkylation Reagents

When used in derivatization for gas chromatography, alkylation represents the substitution of an active hydrogen by an aliphatic or aliphatic-aromatic (benzyl) group. This technique is used to modify those compounds containing acidic hydrogens, such as carboxylic acids and phenols. The principal chromatographic use of this reaction is the conversion of organic acids into esters, which produce better chromatograms than the free acids.

In addition, alkylation reactions can be used to prepare ethers, thioethers and thioesters; N-alkylamines; and amides.



Alkylation of weak acidic groups like alcohols requires strong basic catalysts (sodium methoxide, potassium methoxide). More acidic groups like phenols and carboxylic acids require less basic catalysts (boron trifluoride).

Description	CAS Number	Cat. No.
1,1,1,3,3,3-Hexafluoro-2-propanol, HFIP	920-66-1	02151245
N,N-Dimethylformamide Dimethyl Acetal, DMF-DMA	4637-24-5	02157815
Methoxyamine hydrochloride	593-56-6	02155405
TMAH, etramethylammonium Hydroxide Pentahydrate	10424-65-4	02152114

GC Derivatization Reagents by Application

Derivatization Method	Derivatization Group	Reagent	Cat. No.
Pharmaceuticals, Forensics and Drugs of Abuse			
Silylation	Alcohols	BSTFA	02150476
	Amides		
	Carboxylic Acid		
	Steroids		
		TMSI	02152172
Acylation	Amines	Acetic Anhydride	02154680
		Trifluoroacetic Acid	02300214
	Amides	Trifluoroacetic Acid	02300214
Alkylation	Amines, Amides, Steroids	DMF-DMA	02157815
		TMAH	02152114
Environmental			
Silylation	Steroids	DMF-DMA	02157815
		TMAH	02152114
Food and Beverages			
Silylation	Carbohydrate	BSTFA	02150476
Biofuels			
Silylation		MSTFA	02155675

Generic GC Solvents

MP Bio GC Solvents exhibit the very highest purity and lot-to-lot consistency for gas chromatography applications.

- Contaminant-free to ppb levels
- Meet ACS specifications
- Certificate of Analysis available online
- Chromatograms available upon request

Solvent	Grade	Size	Cat. No.
Acetone	ACS	100 mL	02193832
		500 mL	
		1 L	
		4 L	
n-Hexane	ACS	100 mL	02195220
		250 mL	
		500 mL	
		1 L	
Methanol	HPLC/SPECTRO	1 L	02300141
		4 L	
Methanol Anhydrous	Anhydrous	100 mL	02300138
2-Propanol	Ultra Pure	1 L	02300127
	ACS	1 L	02194007

HPLC Ion Pair Reagents

MP Bio Ion Pair Reagents enable you to quickly and efficiently analyze charged compounds using reversed-phase techniques. Our ion pair reagents are simply dissolved in the HPLC solvent system, resulting in the formation of stable chromatographic complexes that can be separated using reversed-phase columns. By using the correct ion pair reagents, you achieve:

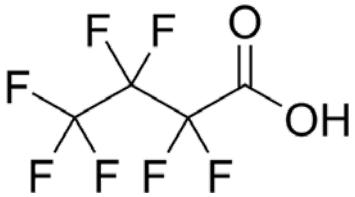
- **Increased or decreased retention, permitting controlled selectivity**
- **Resolution of complex ionic mixtures without using ion exchange columns**
- **Improved peak symmetry**

Heptafluorobutyric Acid

Cat. No. 02151237 | 10 mL, 25 mL, 100 mL

Ion pair reagent for the reversed-phase HPLC separation of proteins and peptides

Heptafluorobutyric Acid is used in determination of amino acid sequences in proteins. It is used as a mobile phase modifier, which significantly improved selectivity in the HPLC analysis of histone proteins. It can be used at a concentration of 0.1% in the mobile phase of an HPLC/LC-MS protocol for the detection of marine bacterioplankton siderophores.



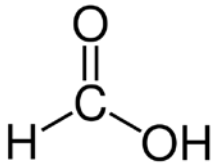
Molecular Formula	$C_4HF_7O_2$
CAS Number	375-22-4
Formula Weight	214.04
Bp	120°C
d	1.64
n_D	1.30 at 20°C
Appearance	Clear, colorless liquid

Formic Acid

Cat. No. 02151162 | 100 mL, 500 mL

Ideal reagent for LC-MS applications

Formic acid is a component commonly found in reversed-phase mobile phases to provide protons for LC/MS analysis. The presence of a low concentration of formic acid in the mobile phase is also known to improve the peak shapes of the resulting separation. Unlike trifluoroacetic acid (TFA), formic acid is not an ion-pairing agent and does not suppress MS ionization of polypeptides when used as a mobile-phase component.



Molecular Formula	CH_2O_2
CAS Number	64-18-6
Formula Weight	46.025
Bp	100.8°C
d	1.22
n_D	1.3714 at 20°C
Appearance	Colorless fuming liquid

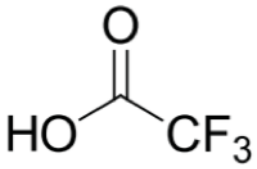
HPLC Ion Pair Reagents

Trifluoroacetic Acid (TFA)

Cat. No. 02300214 | 500 mL, 6 x 500 mL

MP Bio Trifluoroacetic Acid (TFA) is the most commonly used ion pairing agent in reversed-phase peptide separations because TFA:

- Sharpens peaks and improves resolution
- Is volatile and easily removed
- Has low absorption within detection wavelengths



Molecular Formula	$C_2HF_3O_2$
CAS Number	76-05-1
Formula Weight	114.023
Bp	72.4°C
d	1.489
n_D	1.294 at 20°C
Appearance	Clear; Colorless Liquid

Applications:

- Ion pair reagent for reversed-phase HPLC
- Protein/peptide sequencing
- Protein/peptide solubilizing agent
- Solid-phase peptide synthesis
- Amino acid analysis

Ultra Pure Solvents for Amino Acid Analysis

Ideal for HPLC and spectrophotometric applications.

MP Bio HPLC Grade Solvents and Water are specially purified by proprietary methods and tested to ensure lot-to-lot consistency with a low UV absorbance to provide you with the most sensitive detection across all wavelengths. Our solvents are then tested to the highest specifications to ensure the integrity of your data, maximized sensitivity in your assay and prolonged life of your equipment.

Solvent	Grade	Size	Cat. No.
Acetonitrile	HPLC/Spectro	1 L	02300008
		4 L	
Water	Purified, Sterile, DNase & RNase Free	500 mL	04821915
Pyridine	Ultra Pure	100 mL	02151983
		250 mL	
Dimethyl Sulfoxide	ACS	100 mL	02190186
		500 mL	
		1 L	

HPLC Grade Solvents

Solvent	Size	Cat. No.
Acetonitrile	1 L	02300008
	4 L	
Acetone	100 mL	02193832
	500 mL	
	1 L	
	4 L	
1- Butanol	25 mL	02194001
	1 L	
Chloroform, > 99.8%	500 mL	02193814
	1 L	
	4 L	
Cyclohexane	1 L	02300045
Dimethyl Sulfoxide	100 mL	02191418
	500 mL	
	1 L	
Ethyl Acetate, > 99.9%	4 x 4 L	02300084
	4 x 6 L	
Ethyl Ether, > 99.9%, HPLC/Spectro	1 L	02300091
	4 L	
Heptane, > 99.0%	1 L	02300109
	4 L	
	20 L	
Heptane, ACS	1 L	02157323
	100 mL	
Hexane, > 99.0%	6 x 500 mL	02300122
	500 mL	
Hexane	100 mL	02195220
	250 mL	
	500 mL	
	1 L	

Solvent	Size	Cat. No.
Methanol, Anhydrous	100 mL	02300138
Methanol, > 99.8%	1 L	02155387
Methanol, HPLC/Spectro	1 L	02300141
	4 L	
Pentane, Anhydrous	100 mL	02300158
	1 mL	
Pentane, HPLC/Spectro	1 L	02300161
	4 L	
2-Propanol, >99.9%, Ultra Pure	1 L	02300127
	4 L	
2-Propanol, ACS	1 L	02194007
Tetrahydrofuran, > 99.8%, HPLC/Spectro	1 L	02300199
	4 L	
Tetrahydrofuran, > 99.8%, Non UV, HPLC	1 L	02300200
Tetrahydrofuran, Anhydrous	100 mL	02300196
	1 L	
Toluene, > 99.8%, HPLC/Spectro	1 L	02300205
	4 L	
Toluene, > 99.9%, Ultra Pure	1 L	02300202
	4 L	
Toluene, > 99.8%, Anhydrous	100 mL	02300204
	1 L	
1,2,4-Trichlorobenzene, >99.0%, HPLC/Spectro	1 L	02300208
	4 L	
Triethylamine, >99.5%, HPLC	1 L	02300212
Water, HPLC	4 x 4 L	02300223

HPLC Mobile Phase Buffer Preparation Guide

Name	Concentration	Volume or Mass	Preparation Procedure	pH Adjustment Acid/Base	MS Compatible
Acetic Acid	0.1%	1.0 mL	1	-	✓
Ammonium Hydroxide	0.1%	1.0 mL	1	-	✓
	0.2%	2.0 mL	1	-	✓
	1.0%	10.0 mL	1	-	✓
	100 mM	6.9 mL	1	-	✓
Formic Acid	0.05%	0.5 mL	1	-	✓
	0.1%	1.0 mL	1	-	✓
	0.2%	2.0 mL	1	-	✓
	0.5%	5.0 mL	1	-	✓
	50 mM	2.1 mL	1	-	✓
	100 mM	4.2 mL	1	-	✓
Phosphoric Acid	0.1%	1.0 mL	1	-	✓
Trifluoroacetic acid	10 mM	0.8 mL	1	-	✓
	0.1%	1.0 mL	1	-	✓
Acetic Acid Triethylamine EDTA	50 mM	2.8 mL	2	Acetic Acid/ Triethylamine	
	50 mM	6.9 mL			
	2 mM	0.75 g			
Ammonium Formate	10 mM	0.63 g	2	Formic Acid	✓
	15 mM	0.95 g	2	Formic Acid	✓
	100 mM	6.31 g	2	Formic Acid	✓
Ammonium Acetate	10 mM	0.77 g	2	Acetic Acid	✓
	20 mM	1.54 g	2	Acetic Acid	✓
	100 mM	7.71 g	2	Acetic Acid	✓
Hexafluoro Isopropanol Triethylamine	0.4 M	41.5 mL	4	Triethylamine	✓
	16.3 mM	2.3 mL			
Sodium Phosphate, Dibasic	20 mM	2.84 g	2	Phosphoric Acid	

Preparation Procedure 1

1. Add the indicated amount(s) of mobile phase additive(s) to 950 mL of water.
2. Mix solution thoroughly.
3. Measure, adjust and record mobile phase pH (if desired).
4. Add water to final volume of 1 L, degas and transfer to mobile phase container.

Preparation Procedure 2

1. Add indicated amounts of buffers to 400 mL of water and mix thoroughly until all salts are dissolved.
2. Filter solution through a 0.2µm HPLC-certified Nylon filter.
3. Add water to 950 mL and check pH.
4. Adjust pH to desired value.
5. Add water to final volume of 1 L, degas and transfer to mobile phase container.

Preparation Procedure 3

1. Add the indicated amounts of mobile phase buffers to 950 mL of water.
2. Mix TEAA buffer solution thoroughly, measure pH, and adjust pH up ($(CH_3CH_2)_3N$) or down (CH_3COOH) to desired value.
3. Add water to final volume of 1 L. Use this 100 mM TEAA buffer for mobile phase preparation described in step (4).
4. Combine 100 mM TEAA buffer prepared in previous step (3) with organic modifier (e.g., for a 95% 100 mM TEAA/5% ACN mobile phase (v:v), mix 950 mL of 100 mM TEAA buffer with 50 mL ACN).
5. Degas and transfer to mobile phase container.

Preparation Procedure 4

1. Add the indicated amounts of mobile phase buffers to 950 mL of water.
2. Mix buffer solution thoroughly, measure pH, and adjust with TEA if necessary.
3. Add water to final volume of 1 L. Use this buffer for mobile phase preparation described in step (4).
4. Combine buffer prepared in previous step (3) with organic modifier (e.g., for a 95% 0.4 M HFIP, 16.3 mM TEA/5% MeOH mobile phase (v:v), mix 950 mL of buffer with 50 mL MeOH).
5. Degas and transfer to mobile phase container.

Reversed-Phase HPLC Buffers

When samples contain ionizable compounds, the mobile phase pH can be one of the most important variables in the control of retention in a reversed-phase HPLC separation. However, if it is not controlled properly, pH can be a source of many problems. Since most compounds analyzed by RP-HPLC contain one or more acidic or basic functional groups, most mobile phases require pH control. For this reason, buffers are widely used. MP Bio offers a wide selection of high-quality buffers for your reversed-phase HPLC applications.

Common acid-bases used as pH buffer in HPLC

Name	MW	Cat. No.	pK_a	UV cut-off	Recommended Buffer Range
Hydrochloric Acid	36.46	02194054			
Sodium Hydroxide	40.00	02153495			
Trifluoroacetic Acid	114.03	02300214	0.50	210 nm (0.1%)	
Acetic Acid	60.05	02193830	4.76	210 nm (10 mM)	3.76-5.76
Sodium Acetate	82.03	02195496	4.76		3.76-5.76
Potassium Acetate	98.13	02191425	4.76	210 nm (10 mM)	3.76-5.76
Phosphoric Acid	98.0	02300170	2.15 7.20 12.15	< 200 nm (10 mM)	1.15-3.15 6.20-8.20 11.15-13.15
Sodium Phosphate Dibasic	119.98	02199802	2.15 7.20 12.15		1.15-3.15 6.20-8.20 11.15-13.15
Sodium Phosphate Monobasic Monohydrate	159.96	02199202	2.15 7.20 12.15		1.15-3.15 6.20-8.20 11.15-13.15
Potassium Phosphate Dibasic	136.08	02191431	2.15 7.20 12.15		1.15-3.15 6.20-8.20 11.15-13.15
Citric Acid	192.12	04800681	3.13 4.76 6.40	230 nm (10 mM)	2.13-4.13 3.76-5.76 5.40-7.40
Formic Acid	46.02	02151162	3.74	210 nm (10 mM)	2.74-4.74
Sodium Formate	68.01	02152576	3.74		2.74-4.74
Ammonium Hydroxide	35.05	02193854	9.24	200 nm (10 mM)	8.24-10.24
Ammonium Chloride	53.49	02199965	9.24		8.24-10.24
Ammonium Acetate	77.08	02300012	4.76 9.24	205 nm (10 mM)	3.76-5.76 8.24-10.24
Ammonium Formate	63.06	02199646	3.74 9.24		2.74-4.74 8.24-10.24
Tris	121.14	02103133	8.08	205 nm (10 mM)	7.08-9.08
Tris-HCl	157.60	04816116	8.08		7.08-9.08
Triethylamine	157.60	02300212	8.08	< 200 nm (10 mM)	7.08-9.08

Solvents for Histology Applications

Dehydrating Agents

The first stage in tissue processing is dehydration (the removal of water). In tissues, water is present in both free and bound forms and needs to be removed before processing can continue. Dehydration is usually carried out using alcohols (such as ethanol), but these can dissolve certain cellular components such as lipids. Although dehydration can also cause tissue shrinkage, the stage is necessary in all infiltration methods, except where tissues are supported by an aqueous embedding medium (such as water-soluble waxes).

In paraffin wax processing, dehydration from aqueous fixatives such as formalin is usually initiated in 70% alcohol before progressing through 90%-95% to absolute alcohol before proceeding to the clearing stage. However, direct transfer to 95% alcohol is often performed if tissues are adequately fixed. Duration of dehydration is dependent on tissue thickness; the thicker the block, the longer the time. Generally, blocks 1 mm thick should receive up to 30 minutes, while blocks 5 mm thick require up to 90 minutes or longer in each change.

Description	Cat. No.
Acetone Colorless, flammable liquid with a characteristic odor, low toxicity and is freely miscible with water and organic solvents. Ideal for fatty tissue samples and can be transferred directly from acetone into paraffin wax.	02300003
n-Butanol This alcohol is mainly used for plant and animal tissues. N-butanol causes less hardening and shrinkage than ethanol but is poorly miscible with water and paraffin wax, thus requiring longer times.	02194001
Ethylene Glycol Colorless, almost odorless flammable liquid. Miscible with water and most other organic solvents. As a dehydrating agent, it is especially used for preceding ester wax embedding.	02151089
p-Dioxane Colorless, flammable liquid that produces less shrinkage and hardening than with ethanol. The liquid is miscible with water, most organic solvents and paraffin wax and is excellent for tissues that have been excessively hardened by conventional processing. Dioxane has a rapid but gentle action and tissues can remain submerged in it for long periods without harm.	02300081
Ethanol A rapid and efficient dehydrant and the most commonly used. Dehydration is usually initiated in 75% alcohol with progress through 90%-95% ethanol before several changes of absolute ethanol to complete dehydration.	02300010
Isopropyl Alcohol This is completely miscible with water and most organic solvents and is fully miscible with molten paraffin wax. Isopropanol shrinks and hardens tissues and is used to dehydrate hard, dense tissues.	02300128
Methanol Methanol tends to harden tissues more than ethanol and is a poor lipid solvent.	02155387
Tetrahydrofuran Colorless, highly volatile and flammable solvent with an offensive smell. The solution is completely miscible with water, most organic solvents, paraffin wax and mounting media. It dehydrates rapidly causing little shrinkage or hardening and is possibly the best of the universal solvents.	02300198

Solvents for Histology Applications

Clearing Agents

Clearing is the transition step between dehydration and infiltration with the embedding medium. Although tissues are water-free following dehydration, infiltration with wax cannot be carried out because wax and ethanol are largely immiscible. Many dehydrants are immiscible with paraffin wax and a solvent (clearing agent or ante medium) miscible with both the dehydrating agent and the embedding medium is used to assist the transition between these steps. The term clearing arises because some solvents have a high refractive index. When dehydrated tissues are placed into these reagents, they are rendered transparent. This property is used to determine the endpoint and duration of the clearing step, since the presence of opaque areas indicates incomplete dehydration. Clearing agents are fat solvents and therefore remove fat from the tissue. It must be noted that shrinkage occurs when tissues are transferred from the dehydrating agent to the clearing agent and from the clearing agent to wax. In the final stage, shrinkage may result from the extraction of fat by the clearing agent. Xylene is the most popular clearing agent and several changes of it are required to completely displace the ethanol. The choice of a clearing agent depends upon the type of tissue processor used and processing conditions such as temperature, safety factors and cost.

Name	Description	Cat. No.
Amyl Acetate	These are chiefly used as nitrocellulose solvents in double embedding techniques. They have low toxicity, but their strong penetrating odors necessitate good laboratory ventilation. They are ideal for manual processing, as tissues may be left in them for extended periods without hardening.	02106049
Methyl Benzoate		02106645
Methyl Salicylate		02106685
Benzene	Benzene is more gentle and rapid than xylene and toluene and is probably the best transition solvent.	02193879
Butyl acetate	This is used as a xylene substitute and nitrocellulose solvent.	02106121
Chloroform	It causes less brittleness than xylene and is often used on dense tissues such as uterus. However, it attacks some plastics and sealants and is not generally recommended for enclosed processors.	02193814
D-Limonene	This is derived from citrus fruit and is a component of various proprietary blends of transition solvents such as Histoclear and Citroclear, marketed as xylene substitutes.	02155234
Xylene	These agents clear rapidly, and tissues are rendered transparent, facilitating clearing endpoint determination. Concerns over the exposure of personnel to xylene relate mainly to the use of the solvent in coverslipping rather than in processing, and xylene substitutes can be used in these circumstances.	02158692
Toluene		02300203

Water for Specific Applications

Cat. No.	Description	Chromatography	Molecular Biology/PCR	Cell Culture
02300223	Purified water for chromatography	✓		
04821932	DEPC-treated water. RNase & DNase Free		✓	
04821739	DEPC-treated water. RNase & DNase Free		✓	
111201105	Purified water for chromatography	✓		
04821947	Reverse osmosis purified water, sterilized for cell culture			✓
091696054	Purified and sterilized water for cell culture			✓
111007201	DEPC-treated water. RNase & DNase Free		✓	
112450204	Purified RNase & DNase Free water. Not DEPC-treated.	✓	✓	
02FC0005	Water for Injection	✓	✓	✓

Fixation Reagents for Immunohistochemistry

For immunohistochemistry (IHC) to succeed, it is essential that the morphology of the tissues and cells is retained and that the antigenic sites remain accessible to the detection reagents being used.

Fixation plays four critical roles in immunohistochemistry:

- Preserves and stabilizes cell morphology and tissue architecture
- Inactivates proteolytic enzymes that could otherwise degrade the sample
- Strengthens samples so they can withstand further processing and staining
- Protects samples against microbial contamination and possible decomposition

Fixative Formulations for Applications

Sample Type or Antigen	Fixative
Most proteins, peptides and enzymes of low molecular weight	4% (w/v) Paraformaldehyde
	4% (w/v) Paraformaldehyde w/ 1% (v/v) glutaraldehyde
	10% Neutral-buffered formalin (NBF)
Delicate tissue	Bouin's fixative
Small molecules such as amino acids	4% (w/v) Paraformaldehyde w/ 1% (v/v) glutaraldehyde
Blood-forming organs (e.g. liver, spleen, bone marrow); connective tissue	Zenker's solution
Large protein antigens (e.g., immunoglobulins)	Precipitating solutions
Ideal for electron microscopy	4% (w/v) Paraformaldehyde w/ 1% (v/v) glutaraldehyde

Fixative Preparation Guide

4% (w/v) Paraformaldehyde in 0.1 M phosphate buffer

Name	Cat. No.	Amount	Instructions
NaH_2PO_4	02199802	3.2 g	Dissolve NaH_2PO_4 and Na_2HPO_4 in water. Adjust pH to 7.4, then add Paraformaldehyde. Heat mixture to 60°C while stirring and add 1-2 drops of 1 N NaOH to help the paraformaldehyde to dissolve. Cool and filter the solution.
Na_2HPO_4	02194850	10.9 g	
Water	04821932	1000 mL	
Paraformaldehyde	02150146	40 g	

4% (w/v) Paraformaldehyde in 0.1 M phosphate buffer

Name	Cat. No.	Amount	Instructions
Glutaraldehyde, 50% in H_2O	02198595	20 mL	Prepare 4% paraformaldehyde in 0.1 M phosphate buffer, as above. Add Glutaraldehyde solution (50% in H_2O).

Bouin's fixative

Name	Cat. No.	Amount	Instructions
Saturated aqueous picric acid	N/A	750 mL	Mix all components together to form a homogenous solution. Store at room temperature.
Formaldehyde	02300103	250 mL	
Glacial Acetic Acid	02193830	1000 mL	

10% Neutral-buffered formalin

Name	Cat. No.	Amount	Instructions
$NaH_2PO_4 \cdot H_20$	02191442	4 g	Dissolve NaH_2PO_4 and Na_2HPO_4 in water. Adjust pH to 7.4, then add Formaldehyde (40%). Store at 4°C.
Na_2HPO_4	02194849	6.5 g	
Water	04821932	900 mL	
Formaldehyde	02300103	100 mL	

Zenker's solution

Name	Cat. No.	Amount	Instructions
Mercuric chloride	05221706	5 g	Mix thoroughly to dissolve components, add Glacial Acetic Acid right before use. Wash sample for 24 h with distilled water after fixation. Never use metal forceps to handle tissue, as they may corrode.
Potassium dichromate	02156338	2.5 g	
Sodium sulfate	02191444	0.45 g	
Water	04821932	100 mL	
Glacial Acetic Acid	02193830	5 mL	

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