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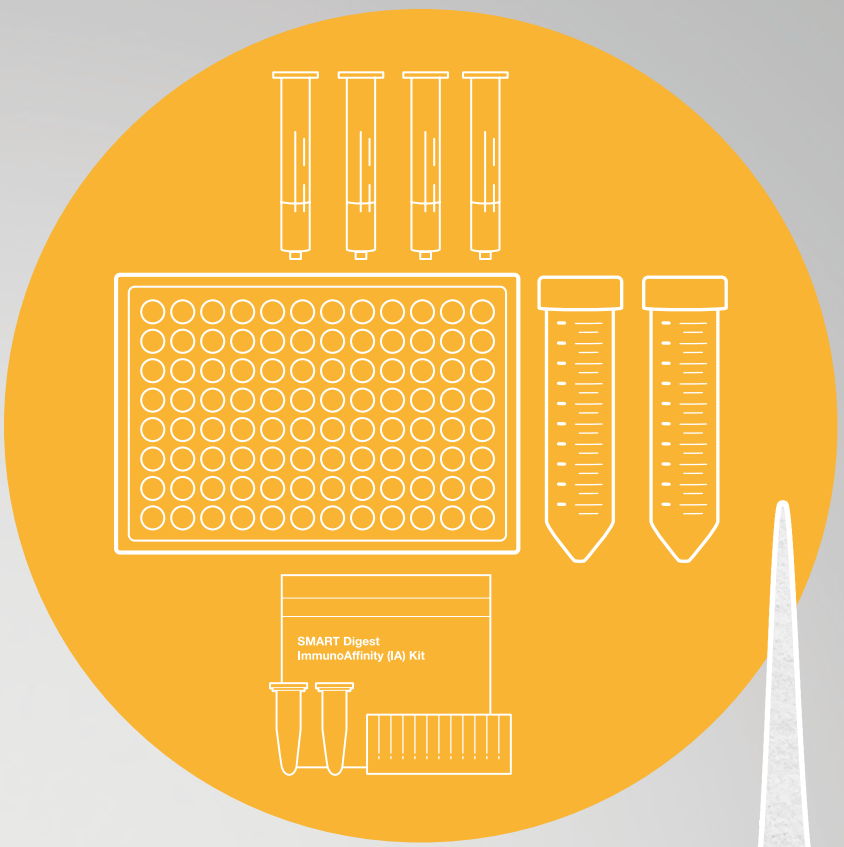


Sample preparation

Sample preparation solutions

Technical resource document

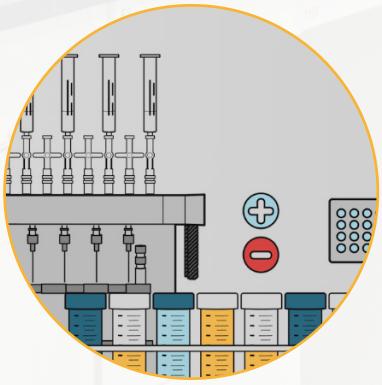
thermo scientific

Introduction

This technical resources document serves as an overview of Thermo Fisher Scientific's sample preparation solutions, providing valuable information for researchers, scientists, and technicians. This document highlights the key features, benefits, and applications of Thermo Fisher Scientific's sample preparation solutions, enabling users to make informed decisions and help optimize their workflows

Whether you are involved in pharmaceutical research, environmental analysis, food safety testing, or any other scientific discipline requiring sample preparation, Thermo Fisher Scientific's sample preparation solutions can help increase productivity, increase the lifetime of other consumables, and streamline your workflows. By leveraging this technical resources document, you can gain a comprehensive understanding of Thermo Fisher Scientific's offerings and bring in the correct products to optimize your workflows and applications.

Sample preparation selection guide



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SPE cartridge selection

Thermo Scientific™ Solid Phase Extraction (SPE) products have been developed to meet the requirements of today's sample preparation challenges. Thermo Scientific SPE cartridges and 96-well plates are offered in a range of phases and bed weights, ideal for use in application areas such as pharmaceutical, biochemical, environmental, and food and beverage. The following flow chart is designed to help in selecting the correct phase for the compound of interest.

Sample matrix	Solubility of sample	Polarity of sample	Separation mode	Recommended Thermo Scientific™ SOLA™, HyperSep™, and Hypercarb™ SPE products
Aqueous	Water soluble	Non-polar	Reversed-phase	SOLA HRP, C18, C8, Phenyl, Retain PEP
		Moderately polar	Reversed-phase	SOLA HRP, C18, C8, Retain PEP
		Polar	Reversed-phase	SOLA HRP, Hypercarb Retain PEP
		Cationic	Ion-exchange	HyperSep SCX
		Anionic	Ion-exchange	HyperSep SAX, HyperSep Aminopropyl
		Non-polar Cationic	Reversed-phase Ion-exchange	SOLA SCX/WCX, Verify CX Retain CX
		Non-polar Anionic	Reversed-phase Ion-exchange	SOLA SAX/WAX, Verify AX Retain AX
Aqueous	Organic soluble	Non-polar	Reversed-phase	SOLA HRP, C18, C8, Phenyl, Retain PEP
Organic	Organic soluble	Polar	Normal phase	HyperSep Silica, HyperSep Cyano, HyperSep Aminopropyl, HyperSep Diol
		Moderately polar	Normal phase	HyperSep Silicia, HyperSep Florisil, HyperSep Aminopropyl, HyperSep Cyano, HyperSep Diol
		Cationic	Ion-exchange	HyperSep SCX
		Anionic	Ion-exchange	HyperSep SAX, HyperSep Aminopropyl
		Non-polar Cationic	Reversed-phase Ion-exchange	HyperSep Verify CX
		Non-polar Cationic	Reversed-phase Ion-exchange	HyperSep Verify AX

SPE phase selection by manufacturer

Thermo Scientific products	Alternative to
HyperSep Retain PEP and **SOLA HRP**	• Waters™ Oasis™ HLB • Phenomenex™ Strata™-X • Agilent™ Bond Elut™ Plexa • Macherey Nagel™ Easy™ • BIOTAGE™ ISOLUTE™ ENV+ SampliQ OPT • UCT™ Styre Screen™ DVB • JT Baker™ H2O-philic DVB
HyperSep Retain CX and **SOLA SCX**	• Waters™ Oasis™ MCX • Phenomenex™ Strata™-X-C • Agilent™ Bond Elut™ Plexa PCX • UCT™ Styre Screen™ DBX • JT Baker™ H_2O-philic SC-DVB
HyperSep Retain AX and **SOLA SAX**	• Waters™ Oasis™ MAX • Agilent™ Bond Elut™ Plexa PAX • UCT™ Styre Screen™ QAX • JT Baker™ H2O-philic SA-DVB
SOLA WCX	• Waters™ Oasis™ WCX • Phenomenex™ Strata™-X-CW
SOLA WCX	• Waters™ Oasis™ WAX • Phenomenex™ Strata™-X-AW
HyperSep Hypercarb	• Millipore Sigma™ Supelclean™ ENVI™-Carb • Agilent™ Bond Elut™ Carbon
HyperSep C18	• UCT™ CLEAN-UP™ C18-U • Supelclean™ ENVI™-18/LC-18 • JT Baker™ BAKERBOND™ SPE™ Polar Plus™ • Biotage™ ISOLUTE™ C18 • Macherey Nagel™ CHROMABOND™ C18 • Agilent™ Bond Elut™ C18 • Phenomenex™ Strata™ C18-U • Waters™ Sep-Pak™ C18
HyperSep C8	• UCT™ CLEAN-UP™ C8 • Millipore Sigma™ Supelclean™ ENVI™-8/LC-8 • JT Baker™ BAKERBOND™ SPE Octyl C8 • Biotage™ ISOLUTE™ C8 • Macherey Nagel™ CHROMABOND™ C8 • Agilent™ Bond Elut™ C8 • Phenomenex™ Strata™ C8 • Waters™ Sep-Pak™ C8
HyperSep Phenyl	• UCT™ CLEAN-UP™ Phenyl • Millipore Sigma™ Supelclean™ LC-Ph • JT Baker™ BAKERBOND™ SPE Phenyl • Biotage™ ISOLUTE™ Ph • Agilent™ Bond Elut™ Ph • Phenomenex™ Strata™ Phenyl (PH)
HyperSep Silica	• UCT™ CLEAN-UP™ Silica • Millipore Sigma™ Supelclean™ LC-Si • JT Baker™ BAKERBOND™ SPE Silica Gel • Biotage™ ISOLUTE™ Silica • Macherey Nagel™ CHROMABOND™ SiOH • Agilent™ Bond Elut™ Si • Phenomenex™ Strata™ Si-1 • Waters™ Sep-Pak™ Si

Thermo Scientific products	Alternative to
HyperSep SCX	• UCT™ CLEAN-UP™ BCX • Millipore Sigma™ Supelclean™ LC-SCX • JT Baker™ BAKERBOND™ SPE Aromatic Sulfonic Acid • Biotage™ ISOLUTE™ SCX • Macherey Nagel™ CHROMABOND™ SA • Agilent™ Bond Elut™ SCX • Phenomenex™ Strata™ SCX
HyperSep SAX	• UCT™ CLEAN-UP™ QAX • Millipore Sigma™ Supelclean™ LC-SAX • JT Baker™ BAKERBOND™ SPE Quaternary amine • Biotage™ ISOLUTE™ SAX • Macherey Nagel™ CHROMABOND™ SB • Agilent™ Bond Elut™ SAX • Phenomenex™ Strata™ SAX
HyperSep Verify CX	• UCT™ CLEAN-UP™ DAU Discovery DSC-MCAX • Biotage™ ISOLUTE™ HCX • Macherey Nagel™ CHROMABOND™ Drug • Agilent™ Bond Elut™ Certify I • Phenomenex™ Strata™ Screen-C
HyperSep Verify AX	• UCT™ CLEAN-UP™ THC • Biotage™ ISOLUTE™ HAX • Agilent™ Bond Elut™ Certify II • Phenomenex™ Strata™ Screen-A
HyperSep Florisil	• UCT™ CLEAN-UP™ Florisil • Millipore Sigma™ Supelclean™ ENVI™-Florisil LC-Florisil • JT Baker™ BAKERBOND™ SPE Florisil • Biotage™ ISOLUTE™ Florisil • Macherey Nagel™ CHROMABOND™ Florisil • Agilent™ Bond Elut™ Florisil • Phenomenex™ Strata™ FL-PR • Waters™ Sep-Pak™ Florisil
HyperSep Aminopropyl	• Uct™ Clean-Up™ Aminopropyl • Millipore Sigma™ Supelclean™ Lc-Nh^2 • JT Baker™ Bakerbond™ Spe Amino • Biotage™ Isolute™ Nh_2 • Macherey Nagel™ Chromabond™ Nh_2 • Agilent™ Bond Elut™ Nh_2 • Phenomenex™ Strata™ Nh_2 • Waters™ Sep-Pak™ Nh_2
HyperSep Cyano	• JT BAKER™ BAKERBOND™ SPE Cyano • MACHEREY NAGEL™ CHROMABOND™ CN • Agilent™ Bond Elut™ Cyano • Waters™ Sep-Pak™ Cyanopropyl • Phenomenex™ Strata™ CN
HyperSep Diol	• Millipore Sigma™ Supelclean™ LC-Diol • JT Baker™ BAKERBOND™ SPE Diol • Macherey Nagel™ CHROMABOND™ OH • Agilent™ Bond Elut™ Diol • Waters™ Sep-Pak™ Diol

Removing uncertainty by applying the science to SPE

Our comprehensive range of SPE solutions offer unparalleled performance in purity of extract and reproducibility. Having a fundamental effect on the quality, time and analysis cost, SPE is a critical step during the sample analysis procedure.

We are dedicated to supplying the highest quality SPE solutions, in combination with providing our customers with the support and resources to optimize their SPE solutions and maximize their analysis.

The importance of SPE

Reducing the effects of the matrix on the separation (GC/LC) and detection (UV, MS, etc.) is beneficial. The use of SPE as a sample preparation technique can significantly improve analysis aiding robustness and generating reproducible accurate, precise and sensitive analytical methods. This relies on the ability of SPE to reproducibly.

Maximize detection selectivity

- Reduce ion suppression
- Reduce protein binding
- Reduce matrix interferences by elimination of matrix and particulates
- Compatibility of solvent with analytical technique

Improve analytical system performance

- Longer column lifetimes
- Less detector maintenance
- Autosampler syringes less likely to block
- Less contamination

Improve sensitivity by concentrating of the analytes

- Lower limits of detection
- More accurate quantitation
- Improved data processing

Matrix effects are an issue in many areas of analytical chemistry, however, modification of ionization (ion suppression/ enhancement) can be a major problem in atmospheric pressure ionization (API) mass spectrometry and, in particular, electrospray based ion sources.

SPE procedure – six steps for a clean extract

1. Sample pre-treatment

It is important to optimize the sample for effective analyte retention. The following should be considered:

- Sample volume/analyte concentration/ matrix complexity
- Adjust sample/matrix composition for proper dilution/ionic strength
- Sample pH for optimum retention
- Confirm that analytes are free in solution
- Remove any unwanted particulates via filtration or centrifugation

2. Column conditioning

Prepare the sorbent for effective interaction(s) with the compounds of interest.

- Use appropriate solvent for column condition/activation
- Prevent sorbent drying during conditioning

3. Column pre-equilibration

Equilibrate with weakly eluting solvent to prepare the phase for sample addition.

- Use the same solvent as for sample pre-treatment
- Prevent sorbent drying during column equilibration

4. Sample loading

Analytes are retained on the sorbent.

- Apply samples at appropriate flow rate (1 mL/minute typical)

For reversed-phase interactions

- Neutral compounds are not affected by pH
- For charged compounds, a pH at which the compound is not charged is used. Neutralize the molecule according to the following:
  - For basic compounds, the neutral molecule exists at least 2 pH units below the pKa of the compound
  - For acidic compounds, the neutral molecule exists at least 2 pH units above the pKa of the compound.

For normal-phase interactions

- pH is not normally an issue in normal phase interactions, as the solvents used are typically non-polar organic solvents, rather than water
- There is no need to verify the sample application pH

For ion-exchange interactions

- pH and pKa are important considerations
- Acidic compounds are extracted from a sample solution at least 2pH units above the pKa of the analyte
- Basic compounds are extracted from a sample solution 2 or more pH units below the pKa of the analyte
- For second (organic) wash, choose the strongest solution where no compound breakthrough occurs
- For elution step, use a solution stronger than where all the compound of interest is eluted
- NB: when choosing these solutions allow some margin for error

5. Wash away interferences

Remove impurities bound less strongly than the compounds of interest.

- Select a strong enough wash solvent to remove interferences but weak enough to leave compounds of interest bound
- Selectively rinse away the less strongly bonded interferences
- Wash solvent selected according to phase mechanism/analyte properties

6. Elute compounds of interest

Selectively recover the analyte(s) by disrupting the analyte-sorbent interaction.

- Selectively elute analytes of interest using different solvents
- Smaller elution volume produces a more concentrated extract
- Select elution solvent that does not elute strongly retained impurities
- Select elution solvent according to phase mechanism/analyte properties

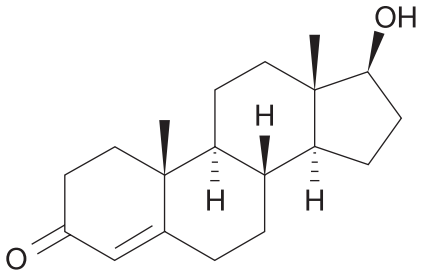
It is important to optimize the Wash and Elution steps in order to obtain maximum levels of recovery.

Method development optimization in SPE

For cleaner extracts, optimization of the SPE process can be important. By optimizing the load, wash, and elution steps of the SPE process, a cleaner sample extract can be obtained.

An example of this is in the development of an SPE method for testosterone. Thermo Scientific™ HyperSep™ Retain PEP SPE cartridge P/N **60107-201**.

1. Condition with 1 mL methanol followed by 1 mL water
2. Load 1 mL of 500 ng/mL sample in water
3. Sequentially wash with increasing strengths of methanol in water, collecting the eluent 0% methanol/100% water to 90% methanol/10% water, increasing methanol content by 10% each time
4. Elution with multiple volumes of methanol



LogP = 3.6 Pk_a = 2.99

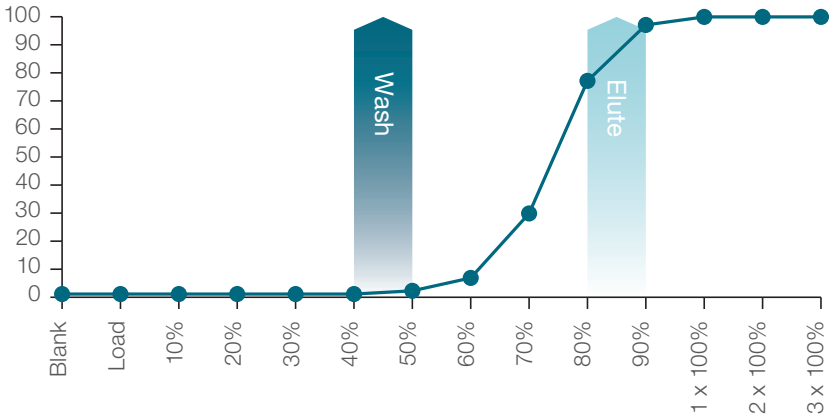


Figure 1. Elution profile of testosterone

By following this procedure the optimum wash and elution steps can be determined. In subsequent analyses a single wash stage would be used at 50% methanol, followed by a single elution step at 90% methanol. This ensures simplicity of the process, optimum recovery and cleanliness of extract.

An alternative would be to use a HyperSep Retain AX SPE cartridge with the added benefit of phospholipid removal.

For more information on how to optimize you SPE methodology and specific applications visit **thermofisher.com/spe**

Syringes and filters

Titan3 and Target2 syringe filters

Sample preparation with Thermo Scientific™ Titan3™ and Thermo Scientific™ Target2™ syringe filters help provide consistent and reliable experimental results. Both these products provide high quality filtration solutions for a range of samples and applications. The premium Titan3 range provides even higher levels of confidence due to the robust design characteristics (burst pressures of 120 psi for the 30 mm range) cleaner extracts due to the inclusion of a pre-filter (most 30 mm products) and ease of membrane selection via the color coded ring.

Membrane selection guide

Choose a filter or membrane based on:

1. Chemical compatibility of the membrane and housing with your sample matrix
2. Size and amount of particulates in the sample
3. Potential interactions (binding) between the membrane and sample components
4. Special considerations such as requirement for pre-filter or inorganic ion certification

Housings

- Titan3 and Target2 filter housings are manufactured from solvent-resistant, low-extractable polypropylene resins specifically selected for wide compatibility with common HPLC sample matrices
- Solutions at temperatures up to 100°C can be filtered using Target2 and Titan3 syringe filters
- Syringe filters can be sterilized by autoclave at 125°C for 15 minutes
- The inlet connection is an enhanced female Luer Lock™ fitting designed for extra security when attached to a Luer Lock syringe
- The outlet fitting is a standard size male Luer-slip fitting for ease of filtrate collection
- Target2 polypropylene syringe filter housings meet the requirements of 21 CFR 177.1520

Syringes and filters

Titan3 and Target2 syringe filters (continued)

This table offers general guidelines for membrane characteristics and compatible applications.

Membrane type	Membrane characteristics	Applications
Cellulose acetate	• Low protein binding, ideal for aqueous-based samples • High protein recovery from filtrate • Lower protein binding compared to PVDF	• Tissue culture media filtration, sensitive biological samples
Glass MicroFiber	• Larger porosity • Able to remove large particulates without clogging	• Dissolution testing, general filtration
Nylon	• Most frequently selected membrane • Broad compatibility with aqueous and organic • Naturally hydrophilic membrane • Extremely low in extractables • Excellent flowrate with most sample matrices • Not compatible with strong acids or bases	• General laboratory filtration • Filtration for most samples • HPLC samples • NOTE: Nylon binds protein, do not use when high protein recovery is desired
Polyethersulfone (PES)	• High flowrates with good throughput volume • Low protein binding • Compatible with high temperature liquids • Mechanically strong membrane low in inorganic extractable ions	• PES is certified for ion chromatography • Tissue culture filtration • Filtration of proteins and nucleic acids
Polypropylene	• Hydrophobic membrane has wide chemical compatibility with organic solvents • Low nonspecific protein binding	• Filtration of biological samples • Filtration of aggressive organic solutions
PTFE	• Hydrophobic membrane is resistant to nearly all solvents, acids, and bases • Membrane is mechanically strong and will withstand exposure to high temperature liquids • Low in extractables • PTFE blocks water vapor • Can be used to filter aqueous solutions after prewetting with an alcohol • The hydrophilic PTFE option provides the same application and performance characteristic, but does not require prewetting of the membrane when filtering aqueous samples	• Filtration of aggressive organic, highly basic or hot solutions, ideal for transducer protectors
PVDF	• Hydrophilic membrane with good solvent resistance • Low UV absorbing extractables and low nonspecific binding	• General biological filtration • Filtration of samples where high protein recovery is desired
Regenerated cellulose	• Hydrophilic membrane with good solvent resistance, extremely low nonspecific binding • Compatible with nearly all common HPLC solvents • Tolerates aqueous samples in pH range of 3 to 12	• Membrane of choice for low nonspecific binding applications • Tissue culture media filtration and general biological sample filtration

Syringes and filters

Syringe filter membrane compatibility chart

Use the information in this table to determine the ability of a specific syringe filter membrane to withstand exposure to a solvent. All concentrations are 100% unless noted.

	Chemicals	NY	PTFE	PVDF	RC	PES	GMF	PP	CA
Acids	Acetic, glacial	LC	C	C	C	C	C	C	IC
	Acetic, 25%	C	C	C	C	C	C	C	C
	Hydrochloric, concentrated	IC	C	C	IC	C	C	C	IC
	Hydrochloric, 25%	IC	C	C	IC	C	C	C	IC
	Sulfuric, concentrated	IC	C	IC	IC	IC	C	C	IC
	Sulfuric, 25%	IC	C	C	LC	C	C	C	IC
	Nitric, concentrated	IC	C	C	IC	IC	LC	C	IC
	Nitric, 25%	IC	C	C	IC	C	LC	C	IC
	Phosphoric, 25%	IC	C	ND	LC	ND	ND	C	C
	Formic, 25%	IC	C	ND	C	ND	C	C	LC
	Trichloroacetic, 10%	IC	C	ND	C	ND	ND	C	C
Alcohols	Methanol, 98%	C	C	C	C	C	C	C	C
	Ethanol, 98%	C	C	C	C	C	C	C	C
	Ethanol, 70%	LC	C	C	C	C	C	C	C
	Isopropanol	C	C	C	C	C	C	C	C
	n-Propanol	C	C	C	C	C	C	C	C
	Amyl Alcohol (Butanol)	C	C	C	C	C	C	C	C
	Benzyl alcohol	C	C	C	C	ND	IC	C	LC
	Ethylene glycol	C	C	C	C	C	C	C	C
	Propolene glycol	C	C	C	C	C	C	C	LC
	Glycerol	C	C	C	C	C	C	C	C
Amines and amides	Dimethyl formamide	LC	C	IC	LC	IC	C	C	IC
	Diethylacetamide	C	C	ND	C	ND	C	ND	IC
	Triethanolamine	C	C	ND	C	ND	ND	ND	C
	Aniline	ND	C	ND	C	ND	ND	ND	IC
	Pyridine	C	C	IC	C	IC	C	IC	IC
	Acetonitrile	C	C	C	C	LC	C	C	IC
Esters	Ethyl acetate/methyl acetate	C	C	C	C	IC	C	LC	IC
	Amyl acetate/butyl acetate	C	C	IC	C	IC	C	LC	LC
	Propyl acetate	C	C	IC	C	IC	ND	LC	LC
	Propylene glycol acetate	ND	C	ND	C	IC	ND	C	IC
	2-Ethoxyethyl acetate	ND	C	ND	C	IC	ND	ND	LC
	Methyl cellusolve	ND	C	ND	C	IC	C	C	IC
	Benzyl benzoate	C	C	ND	C	IC	ND	ND	C
	Isopropyl myristate	C	C	ND	C	IC	ND	ND	C
	Tricresyl phosphate	ND	C	ND	C	IC	ND	ND	C

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	Chemicals	NY	PTFE	PVDF	RC	PES	GMF	PP	CA
Halogenated Hydrocarbons	Methylene chloride	LC	C	C	C	IC	C	LC	IC
	Chloroform	C	C	C	C	IC	C	LC	IC
	Trichloroethylene	C	C	C	C	IC	C	C	C
	Chlorobenzene	C	C	C	C	LC	C	C	C
	Freon	C	C	C	C	LC	C	C	C
	Carbon tetrachloride	C	C	C	C	IC	C	LC	LC
Hydro-carbons	Hexane/xylene	C	C	C	C	IC	C	IC	C
	Toluene/benzene	C	C	C	C	IC	C	IC	C
	Kerosene/gasoline	C	C	C	C	LC	ND	LC	C
	Tetralin/decalin	ND	C	C	C	ND	ND	ND	C
Ketones	Acetone	C	C	IC	C	IC	C	C	IC
	Cyclohexanone	C	C	IC	C	IC	C	C	IC
	Methyl ethyl ketone	C	C	LC	C	IC	C	LC	LC
	Isopropylacetone	C	C	IC	C	IC	C	ND	C
	Methyl isobutyl ketone	ND	C	LC	C	IC	C	LC	ND
Organic Oxides	Ethyl ether	C	C	C	C	C	ND	LC	C
	Dioxane	C	C	LC	C	IC	C	C	IC
	Tetrahydrofuran	C	C	LC	C	IC	C	C	IC
	Triethanolamine	C	C	ND	C	ND	ND	ND	C
	Dimethylsulfoxide (DMSO)	C	C	IC	C	IC	C	C	IC
	Isopropyl ether	ND	C	C	C	C	ND	C	C
Miscellaneous	Phenol, aqueous solution, 10%	ND	C	LC	IC	IC	C	C	IC
	Formaldehyde aqueous solution, 30%	C	C	C	LC	C	C	C	C
	Hydrogen peroxide, 30%	C	C	ND	C	ND	ND	ND	C
	Silicone oil/mineral oil	ND	C	C	C	C	C	C	C
	Ammonium hydroxide, 25%	C	C	LC	LC	C	C	C	C
	Sodium hydroxide, 3N	C	C	C	LC	C	IC	C	IC

Legend	
C	Compatible
LC	Limited compatibility (Membrane may swell and shrink)
IC	Incompatible (Not recommended)
ND	No compatibility data currently vailable
PTFE	Polytetrafluoroethylene
PVDF	Polyvinylidene fluoride
PES	Polyethersulfone
CA	Cellulose acetate
RC	Regenerated cellulose
PP	Polypropylene
GMF	Glass MicroFiber

Learn more at **thermofisher.com/chromsampleprep**

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