



OPERATING MANUAL

RESOLVEX™ M10 96

and

RESOLVEX™ M10 96 XT

MULTI-CHANNEL SPE PROCESSOR



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1 Product Overview

The RESOLVEX™ M10 96 and RESOLVEX™ M10 96 XT (Extra Tall) for Positive Pressure Solid Phase Extraction (SPE) offers state-of-the-art operation for SPE in 96-well formats.

The main difference between the standard M10 96 configuration and its M10 96 XT counterpart is that in the XT model, the height travel range of the pressure manifold has been extended by 0.954" (2.42 cm.) to accommodate taller column stacks.

The configuration setup that the end-user intends to use, will dictate which M10 Processor to use.

Typically:

- If you intend to use a 96-Place rack with regular-sized collection plates, you will need the XT
 - These 96-Place racks are intended to be used with 95 or less columns.
- All other users will need the standard M10 96 configuration

For simplicity, the rest of this operating manual will refer to both product configurations as the RESOLVEX M10 96

Positive pressure processing of SPE columns exhibits a number of advantages compared to traditional vacuum manifold processing.

One major problem with conventional vacuum manifolds occurs as faster flowing columns on the manifold run dry, allowing a free flow path for vacuum through the dry columns, slowing the flow on the remaining columns. This results in additional variation in column processing times, thus contributing to irreproducible analyte recoveries. Also, when running fewer columns than needed to accommodate all manifold ports, open ports must be plugged or switched off to prevent vacuum loss, which otherwise causes insufficient flow through the sample columns.

In contrast to vacuum manifolds, the RESOLVEXTM M10 96 uses unique, restricted gas ports. These ports cause gas flow control such that even if 95 processing channels are wide open, the remaining single channel will receive appropriate pressure to allow facile processing. This feature provides very uniform flow across all column channels, and eliminates the need for plugs of caps when processing fewer than 96 samples.

When processing viscous samples, vacuum manifolds often cannot provide adequate processing power since the maximum pressure is limited to something less than atmospheric (≈15 PSI/1.03 bar). The RESOLVEX™ M10 96 allows for gas pressures up to 5.5 bar (80 PSI), supplying greater motive force for viscous sample flow. If the user adjusts the pressure up or down, the column flow response is smooth and immediate.

The RESOLVEX™ M10 96 is self-adjusting to column height, allowing it to accommodate all commercial 96-well plates, both modular and integrated, without the need for special adapters or accessories.



2 Safety

2.1 Intended Use

The RESOLVEX™ M10 96 will be used to perform manual solid phase extraction of analytes from various biological sample types as a stand-alone, independent module for sample preparation. The product is intended to be used in a professional laboratory setting to perform various manual solid phase extraction procedures using positive pressure application to 96-well format filter/extraction plates to prepare samples for downstream analysis (*e.g.* LC/MS, HPLC).

2.2 Safety Considerations

Many reagents and samples used with the RESOLVEX[™] M10 96 can pose chemical, biological and radiological hazards. The user must always understand the potentially hazardous effects of all the materials he/she works with. To prevent personal injury and/or equipment damage, we recommend that the user always use impermeable gloves, chemical safety goggles, and protective clothing following GLP (Good Laboratory Practice). Consult your company's safety expert for guidance. The RESOLVEX[™] M10 96 are manufactured from aluminum, stainless steel, and plastics. Always clean up spills or overflow immediately to prevent any damage to the equipment.

Warning: "This product can expose you to chemicals including Lead, which is known to the State of California to cause cancer, and Bisphenol A, which is known to the State of California to cause birth defects or other reproductive harm. For more information go to www.P65Warnings.ca.gov."

2.3 Disposal

Follow laboratory procedures for biohazardous waste disposal according to national and local regulations

This chapter provides instructions for how to lawfully dispose of waste material accumulating in connection with the Tecan RESOLVEX M10 48



CAUTION

OBSERVE ALL FEDERAL, STATE AND LOCAL ENVIRONMENTAL REGULATIONS

2.3.1 Disposal of Packing Material

The packing material consists of recyclable material. If you do not intend to keep it for future use, e.g. for transport and storage purposes, please dispose of the packing material according to local regulations.



2.3.2 Disposal of Operating Material





BIOLOGICAL HAZARDS CAN BE ASSOCIATED WITH THE WASTE MATERIAL (MICROPLATE) OF THE PROCESS RUN ON THE TECAN RESOLVEX M10
TREAT THE USED MICROPLATE, OTHER DISPOSABLES, AND ALL SUBSTANCES USED IN ACCORDANCE WITH GOOD LABORATORY PRACTICE GUIDELINES. INQUIRE ABOUT APPROPRIATE COLLECTING POINTS AND APPROVED METHODS OF DISPOSAL IN YOUR COUNTRY, STATE, OR REGION.

2.3.3 Disposal of the unit

If you have any questions concerning the disposal of the unit, please contact your local Tecan customer support.

Pollution degree	2 (IEC/EN 61010-1)
Method of disposal	Contaminated waste

2.4 Applicable Symbols

Symbol	Definition
Tecan SP, Inc. 14180 Live Oak Avenue, Baldwin Park, CA 91706 USA	Indicates the manufacturer
<u> </u>	Indicates the need for the user to consult the instructions for use
M	Indicates the date when the unit was manufactured
REF	Indicates the manufacturer's catalogue/part number
SN	Indicates the manufacturer's serial number so that a specific unit can be identified
<u></u>	Indicates the range of humidity to which the unit can be safely exposed
1	Indicates the temperature limits to which the u can be safely exposed
\square	Indicates the date after which the product is not to be used



2.5 Warning Symbols

The following warning symbols are to alert the user of the risks (of death, injury, or physical reactions) that may arise if the unit is used or misused.

Symbol	Definition
\triangle	Indicates the need for the user to consult the instructions for use for important cautionary information such as warnings and precautions that cannot, for a variety of reasons, be presented on the unit itself
	Indicates a warning of a closing motion of mechanical parts of equipment. Care should be taken to avoid injury to hands when in the vicinity of equipment with closing mechanical parts.
	Biological Risk



WARNING

DEPENDING ON THE APPLICATIONS, PARTS OF THE TECAN RESOLVEX M10

MAY HAVE BEEN IN CONTACT WITH BIO-HAZARDOUS MATERIAL.

- MAKE SURE TO TREAT THIS MATERIAL ACCORDING TO THE APPLICABLE SAFETY STANDARDS AND REGULATIONS.
 - DECONTAMINATE ALL PARTS BEFORE DISPOSAL.

2.6 **Laboratory Practices**

Warning: Extreme care should be exercised when handling flammable solvents to prevent physical damage to the laboratory and to prevent injury to the users. Material Safety Data Sheets (MSDS) should be available, studied, and carefully followed before using any of the chemicals. Suitable education or training in the handling of chemical is highly recommended.



2.7 Site Preparation

The RESOLVEX™ M10 96 unit should be placed in a laboratory and operated where there is proper ventilation or from inside a chemical fume hood when volatile solvents are used. The RESOLVEX™ M10 96 unit requires a clean, oil-free gas source and must also be located near the installation site.

3 Gas Requirements

The RESOLVEX™ M10 96 operates using compressed air or nitrogen both to seal the columns and to displace liquid.

Warning: It is essential to use a gas source that is free of moisture, particulates, and hydrocarbons to prevent sample contamination and general fouling of the manifold.

The optimum gas supply (5.5 bar/80 PSI) is achieved by a regulator positioned between the gas source and the unit. This unit is supplied with an installation kit which includes 8 feet of 1/8" ID (2.44 Meter of 3.2mm ID) (1/4" (6.35mm) OD) polyethylene tubing and connectors to 1/8" (3.175mm) NPT or 1/4" (6.35mm) NPT.

Pressure Range: Flow 4.1 bar (60 PSI) – 7.0 bar (100 PSI) Max

Optimum conditions: 5.5 bar (80 PSI)

Warning: Gas pressure must not exceed 7.0 bar (100 PSI). Exceeding this pressure may result in damage to the unit.

The installation of an inline pressure regulator, to properly display the line pressure going into the unit and to regulate the inlet line pressure, is highly recommended. If multiple pieces of equipment are connected on the same line, it is encouraged to add an easily accessible shut-off valve to each of the units, should disconnection be required.

4 Table/Bench and Space Requirements

For the RESOLVEX™ M10 96 to function properly, adequate spacing and table support is required. The minimum space required is 10.2" (259mm) W x 12.0" (305mm) D x 15.4" (390mm) H. The free space above and behind the bench should be at least 4" (10cm) for ventilation. A flat area, stable and able to support the weight of the unit (26 lbs./11.6 kg) should be used.

4.1 Environmental Conditions

The RESOLVEX [™] M10 96 is intended for indoor use only with an ambient surrounding temperature range of 15°C to 32°C (59°F to 90°F).

The relative humidity (non-condensing) should be 30% to 80%.

5 Unpacking the RESOLVEX™ M10 96

The total weight of the package is approximately 35 lbs./16 kg and good safety practices should be performed when handling, moving, and unpacking the shipping boxes.



The RESOLVEX™ M10 96 is typically shipped with the following items:

- 296-7501 RESOLVEX™ M10 96
- 278-2036 Operation Manual
- 279-0010 Installation Kit
- 278-0035 System 96 Column Seal, Removable Adhesive (6 month shelf life)
- 278-0021 96 Deep Well Collection Plate, 1.2mL x 96-Well
- 278-0020 96-Well x 2mL Collection Plate
- 278-0300 Single Well Plate, 96 Well Reservoir, No Dividers 300mL Volume

The RESOLVEX™ M10 96 XT (Extra Tall) Option is typically shipped with the following items:

- 288-0006 RESOLVEX™ M10 96 XT
- 278-2036 Operation Manual
- 279-0010 Installation Kit
- 278-0035 System 96 Column Seal, Removable Adhesive (6 month shelf life)
- 278-0012 1.2 mL x 96 Well Collection Tray
- 278-0020 96-Well x 2mL Collection Plate
- 278-0030 Single Well Plate, 96 Well Reservoir, No Dividers 300mL Volume
- 278-0302 96-Well Reservoir Waste Tray

6 Installing the RESOLVEX™ M10 96

The RESOLVEX™ M10 96 does not include a filter on the gas supply input. A clean, oil-free gas source must be used to prevent the unit's pneumatics and sample contamination.

Slight movement of the manifold may occur as the unit is pressurized when connecting the gas supply.

- 1. Open the unit box and remove the accessories boxes. Standard contents are listed above.
- 2. Remove the unit from the box and place in desired location. See the Site Requirements in previous page.

Plumb the unit into the gas source by using the provided tubing in the Installation Kit and inserting one end of the tubing into the open port at the rear of the unit and the other end into the supplied gas source. Gas requirements are mentioned in the previous page.



7 Product Warranty

The RESOLVEX™ M10 96 is warranted for 90 days from the date of installation that the unit will be free from defects in materials and workmanship. Warranty repairs will be made given that the product has not been abused or tampered with by persons other than repair personnel authorized by Tecan SP. It is constructed primarily of stainless steel and anodized aluminum. Other materials include silicone rubber, polyethylene, and polypropylene. The controls and gauges are not solvent resistant. Some components, including the column seal, may be replaceable under the warranty. For out-of-warranty repairs, the cost of replacement materials, service time and shipping (if applicable) will be billed accordingly. Should there be any technical problems with the RESOLVEX™ M10 96, please contact Tecan SP Technical Support at (+1) 626-962-0010 (option 5) for further assistance.

Spare parts or replacements beyond the warranty period for such components as collection trays, manifold column seals, and plumbing items are available. Contact Tecan SP Customer Service for current options and pricing at (+1) 626-962-0010 (option 3).

Should the RESOLVEX™ M10 96 need to be returned or shipped back to Tecan SP, a Certificate of Decontamination form must be submitted prior to shipping the unit. Please contact Tecan SP Customer Service at (+1) 626-962-0010 (option 3) for more information on returning or shipping the unit to Tecan SP.



8 Tray Configurations

The RESOLVEX™ M10 96 provides the means to batch process solid phase extraction columns in the 96-position format. The RESOLVEX™ M10 96 will accommodate both the integrated and the modular SPE plates stacked onto a variety of collection plates. The modular SPE plate is a CEREX® Column Holder plus plug-in columns (Figure 1). Rimless CEREX® 1cc columns have been especially designed to fit into the 96-position footprint by insertion into the CEREX® Column Holder.

The re-usable CEREX® Column Holder interfaces to several collection plates which are also in the 96-position format. Depending upon the volume of the effluent, the appropriate collection tray can be selected from the ancillary products available.

The CEREX® Column Holder is keyed to the collection plates so that sample position is preserved throughout the SPE sequence. Both the CEREX® Column Holder and collection plates are keyed to match so that when they are correctly aligned, the assembly sits level on the slide tray platform.



Figure 1. Integrated *CEREX*® plate and collection plate assembly (left), Modular *CEREX*® SPE plate and collection plate assembly (right), Rimless *CEREX*® 1cc columns (center)



9 Column Compression and Decompression

The RESOLVEX™ M10 96 is designed to accept up to 96 columns, installed in a modular plate. Because of the unique manifold design, each column/well is pressurized to the same level even if columns are omitted.

Place the column and tray configuration on the RESOLVEX™ M10 96 slide platform. Slide the platform to the rear of the until it reaches the stop locating it under the manifold.

The two toggle switches located on opposite sides of the enclosure are used to activate the compression mechanism. Both switches must be pressed simultaneously to open or close the compression mechanism. Activate the column compression mechanism to lower the manifold by depressing the bottom portion of both switches simultaneously until the manifold has stopped moving (≈2 to 3 seconds). The switches must be held until the manifold has settled on the configuration. If the switches are released prematurely, the manifold mechanism will automatically open. This is a safety feature designed to keep hands and fingers clear of moving parts during the compression.

Decompression of the rack assembly is activated similarly. Depress the top portion of both switches simultaneously until the manifold is all the way up and has stopped moving. Slide the column and tray configuration forward until it reaches the stop and proceed with the next step of the SPE method.

The compression speed is pre-set and is not adjustable. The pressure applied to the compression system is pre-set and is not adjustable.

10 Column Stacking Option for Positive Pressure Sample Processing

Tecan SP's column stacking for Positive Pressure sample processing (PN 253-0009 and PN 253-0007) offers numerous options and techniques for sample preparation.

- The upper column may be used as a filtration device, as a reaction vessel, or as a solid phase extraction medium.
- Column stacking is compatible with both conventional CEREX® 1mL SPE columns and CEREX® Narrow Bore (NBE) extraction columns.
- CEREX® Controlled Flow Plates (CFP) are used as reaction vessels for tryptic digests. The resulting digests may be eluted into collection plates or subjected to SPE for enrichment/purification.
- Sequential Solid Phase Extraction (SSPE) perform a solid phase extraction process in the upper column and deliver the sample eluate directly to the lower column for secondary extraction using an orthogonal process.
- Column stacking provides direct transfer of samples from one modality to another. Offline sample transfer steps are eliminated.
- Stacking plates may be used in both partial-plate and full-plate modes, providing flexibility in R&D and scalability to production level. Stacked plates are compatible with Tecan's RESOLVEX M10 96 processors.



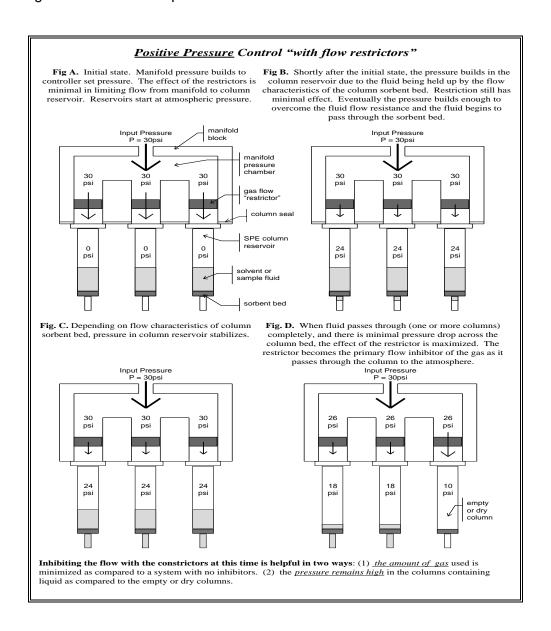
• The use of stacking plates is automatable on Tecan's RESOLVEX A100 Stand-Alone Workstations and on industry-standard robotic liquid handlers.





11 Manifold Operation

Each manifold port is flow-restricted in order to maintain uniform manifold pressure in all positions, even if some positions are not filled by columns. With no column in place, there is approximately 0.75 SCFH (0.35 +/- .236 liter per minute) of flow through a port at 25 PSIG (1.7 bar). The principle of the restrictor manifold design is discussed in the following diagram. The adjustable flow reading on the rotameter equates to the total flow to the turned on rows.





12 Pressure Application

The RESOLVEX™ M10 96 has a two-level gas delivery system used to pressurize the SPE columns. It is user-controlled and operated manually by adjusting the pressure and flow each time the unit is used.

One part of the system is controlled by the rotameter located on the left side of the unit panel. By switching the "FLOW CONTROL" selector to the left, "ADJUSTABLE FLOW" setting, the gas is delivered to the SPE Column Manifold through the ADJ FLOW regulator and then through the rotameter which is controlled by the needle valve located at its base. The rotameter route provides a very precise slow flow through the SPE columns by limiting the gas flow to the manifold from 0 to 2.5 SCFH (1.8 Liters per minute).

NOTE: The rotameter needle valve is for adjustment only and is NEVER to be used as an on off valve. Use the selector valve to turn off the nitrogen flow.

The second part of the gas delivery system provides for rapid gas flow to the manifold which is controlled by adjusting the regulator located below the pressure gauge labeled "MAXIMUM FLOW." The pressure range is 0-7.0 bar (0-100 PSI) and is used to maximize flow through the SPE columns for processing viscous or high particulate samples or to dry the columns in situ. By switching the "FLOW CONTROL" selector to "MAXIMUM FLOW", gas is introduced to the manifold at the pressure set on the "MAXIMUM FLOW" regulator as indicated by the gauge located directly above it.

By turning the "FLOW CONTROL" selector to off, no gas will flow to the manifold; it should be in the off position when decompressing the column set between the various SPE method operations.

Note that the regulator is of the "locking" type. The regulator can be adjusted by pulling out on the regulator knob. Once the desired pressure is set, it can be "locked in" by pushing in on the knob.

13 Waste Management

It is highly recommended to verify the liquid level in the waste/collection tray, and if necessary, properly dispose of the content before the next pressure application cycle begins.

14 Preventive Maintenance

The RESOLVEX™ M10 96 Pressure is a rugged unit constructed of anodized aluminum, stainless steel, and solvent resistant plastics. However, the following daily and periodic maintenances should be observed. The column seal must be inspected daily for proper function of the unit. Failure to observe for detachment from manifold (peeling) and holes or rips in the column seal, may result in improper unit functionality.



14.1 **Daily Maintenance**

- 1. Inspect the manifold column seal for wear and tear or detachment. Replace if necessary or clean using a lint-free cloth or wipe.
- 2. Wipe up all spills on the surface areas of the unit.



Warning: Waste bin and surroundings may be contaminated with biological samples. Operators should wear gloves, protective clothing and follow good laboratory practices when emptying waste bin or cleaning surroundings to prevent exposure to biohazards. Waste should be treated as biohazard materials.

14.2 Periodic Maintenance

- The deck of the RESOLVEX™ M10 96 can become dusty or dirty from solvent or sample spills. Periodic cleaning should take place to prevent damage to the surface areas of the platform.
- 2. A lint-free cloth should be used along with isopropanol and/or a mild cleaning solution to clean the sheet metal and anodized areas.

14.3 **Quarterly Maintenance**

It is recommended to replace the manifold's column seal every 12 months.

14.3.1 Manifold Column Seal Replacement

- 1. Clear sliding tray of collection plates.
- 2. Use the enclosed 3/16" hex key to remove the two 1/4-20 screws fastening the manifold to the unit.
- 3. Lift the manifold off the support rods and place it upside down on the table.
- 4. Remove old column seal from the manifold.
- 5. Remove the backing of the replacement column seal. Care should be taken as to not apply any unwanted dirt or debris on the adhesive side of the column seal.
- 6. Align the holes on the column seal with the manifold holes and adhere.
- 7. Secure the manifold to the support rods with the two ¼-20 screws and hex key. The column seal should be facing down and the manifold tubing toward the rear.

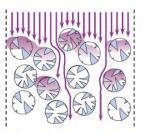


CEREX SPE Columns "Simply Superior SPE"

15 Cerex® Technology

Cerex[®] is a patented manufacturing technology that enables creation of very high performance Solid Phase Extraction (SPE) columns. Cerex[®] technology provides for the use of microparticulate sorbents in the SPE column, typically in the 10µm particle size range, as compared to the 40µm particle size range used by most commercial suppliers. Microparticulate sorbents offer tangible and significant benefits compared to larger particle sorbents; specifically, very high capture efficiency.

Capture efficiency refers to the ability of an SPE device to effectively retain, or "trap" desired analytes from the applied sample. Regardless of the chemical affinity of the sorbent for the analyte species, SPE products that use large particle sorbents may exhibit significant analyte breakthrough during sample application. This is caused by the relatively large flow paths between sorbent particles, allowing analytes to bypass contact with the sorbent, thus obviating retention (Figure 1).



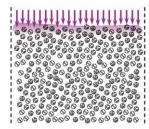


Figure 2 - 10 Micron Path

In contrast, Cerex® microparticulate sorbents *Figure 1 - 40 Microp Path* contain much smaller flow paths, ensuring contact with the sorbent as the analyte molecules pass through the sorbent bed. This results in more efficient and consistent analyte trapping, leading to higher analyte recoveries and more reproducible results (Figure 2).

A simple loading experiment demonstrates the difference between Cerex® microparticulate SPE and a

conventional product containing 40µm sorbent, using surface-matched sorbent chemistries (Figure 3). Breakthrough of a UV-absorbing analyte from each respective SPE column is measured using a conventional UV detector. The trace begins with the SPE column bypassed; therefore, the detector signal

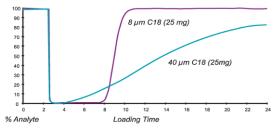


Figure 3 - Cerex Microparticulate SPE vs. 40 Micron Sorbent

reflects the level of analyte in the loading solvent. As the cartridge is placed into the sample stream, absorbance drops initially, and then recovers as analyte "breaks through" the column. Note that the Cerex® column captures roughly 4 times the analyte quantity as the 40µm column before breakthrough, and reaches total saturation capacity immediately thereafter, as seen by rapid recovery of the detector signal to initial conditions. In contrast, the 40µm SPE product never reaches saturation capacity, even after many minutes of sample loading.



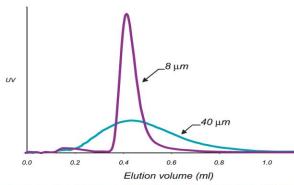


Figure 4 - Chromatogram 10 Micron vs. 40 Micron Elution

This high capture effect allows the use of much smaller bed sizes in Cerex® columns compared to conventional sorbents. For example, a 35mg, or even a 20mg Cerex® column, typically exhibits the same effective capacity as a 100mg, 40µm SPE product. The use of smaller sorbent beds allows for commensurately smaller analyte elution volumes. The benefit of a smaller bed mass on elution volume is in addition to the narrower analyte band width for smaller particles (Figure 4).

16 Product Selection

Cerex[®] SPE products are available in a wide range of sorbent chemistries and configurations. The Cerex[®] chemistry range includes non-polar, polar, and ion-exchange sorbents based on a silica-based substrate. For mixed-mode extraction, silica-based blended phases are available in a selection of both non-polar/cation exchange and non-polar/anion-exchange chemistries. In addition, a selection of water-wettable polymer-based sorbents with non-polar, ion-exchange, and mixed mode characteristics complements the silica-based products.

Column formats offered include standard syringe-barrel tubes with luer-tips in the most widely used sizes (1, 3, and 6 cc tubes). In 96-well formats, both "integrated" (single plate) and "modular" (base plate plus plug-in columns) formats are available to accommodate specific user applications.

Column bed sizes range from 10mg up to a gram scale in the largest columns.

17 Abbreviations

The following abbreviations are provided as a reference and may appear in the Instruction for Use.

°C	Degrees Celsius
CE	Conformité Européenne
CFR	Code of Federal Regulations
cm	Centimeter
EC	European Community
ELISA	Enzyme-linked Immunosorbent Assay
EN	European Norm
°F	Degrees Fahrenheit
FDA	Food and Drug Administration
HPLC	High-performance liquid chromatography
ID	Identification
IFU	Instructions for Use
IQ	Installation Qualification
IVD	In vitro diagnostics
kg	Kilogram
LC-MS	Liquid chromatography – mass spectrometry
1	Liter
mg	Milligram



ml	Milliliter
mm	Millimeter
MTP	Microplate
μl	Microliter
NFM	Nonferrous Metal
NIST	National Institute of Standards and Technology
nm	Nanometer
OD	Optical Density
OQ	Operational Qualification
RoHS	Restriction of the Use of Certain Hazardous
	Substances
SOP	Standard Operating Procedure
UA	Arbitrary Units
TÜV	Technischer Überwachungsverein
	(Technical Inspection Agency)



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