GyroMark™ HT Human Insulin

Gyros Gyrolab™ xP Workstation Assay

Cat. # GYHINS-14K

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INTRODUCTION

The Human Insulin GyroMark™ HT Kit was developed for use on the Gyros Gyrolab™ xP Workstation. This fully automated compact disc-based nanotechnology platform uses microfluidic technology for the sandwich based immunoassay analysis of the GyroMark™ HT kits using a biotinylated capture antibody and a fluorescence labeled detection antibody. The Gyros technology has features including small sample volume (1000 nL), high sensitivity, broad dynamic range, and short assay time (~ 1 hour per assay) giving higher throughput with less sample and less "hands on" lab time. Up to 5 compact discs of samples can be used in one run, allowing for 5 hours of hands-free sample analysis of up to 480 data points. The self-contained data reduction software fully analyzes all results in minutes, including standard curve graphing, raw data, calculated data, %CVs, signal to background, and much more, saving the researcher considerable time spent on data reduction and summarization. EMD Millipore is proud to partner with Gyros AB in bringing you the GyroMark™ HT line of immunoassay kits as an additional tool in helping meet your research needs.

INTENDED USE

The Human Insulin GyroMark™ HT kit is used for the non-radioactive quantification of Insulin in human serum and plasma samples using the Gyros Gyrolab™ xP Workstation. One kit is sufficient to measure 37 unknown samples in duplicate.

This kit is for Research Use Only. Not for Use in Diagnostic Procedures.

PRINCIPLES OF ASSAY

This assay is a sandwich ELISA based, sequentially, on: 1) binding of Insulin biotinylated capture antibody to the streptavidin coated affinity columns of the Bioaffy 1000 nL CD, 2) capture of Insulin molecules from samples to the anti-Insulin antibody, 3) binding of a second dye-labeled anti-Insulin detection antibody to the captured molecules, 4) quantification of Insulin using the Gyrolab™ Evaluator.

REAGENTS SUPPLIED

Each kit is sufficient to run one 96-segment 1000 nL CD and contains the following reagents:

Note: Store all reagents at 2-8°C

Reagents Supplied	Catalog Number	Volume	Quantity
Bioaffy 1000 nL CD	P0004253	---------	1 CD
Human Insulin Standard	GYHINS14-S	Lyophilized	1 vial
Human Insulin Quality Control 1	GYHINS14-QC1	Lyophilized	1 vial
Human Insulin Quality Control 2	GYHINS14-QC2	Lyophilized	1 vial
Human Insulin Quality Control 3	GYHINS14-QC3	Lyophilized	1 vial
Diluent GM3	GMDIL-3	2.5 mL	1 bottle
Human Insulin Capture Antibody	GYHINS14-C	100 µL	1 vial
Human Insulin Detection Antibody	GYHINS14-D	25 µL	1 vial
Serum Matrix	GMSM-4	Lyophilized	1 vial
Detection Diluent	GMDTDIL	200 µL	1 vial
pH 11 Wash Buffer	P0020087	Lyophilized	1 vial
Mixing Bottle	----------	---------	1 vial
Sample Plates/Foil Seals	GM-PLATE	---------	2 each

STORAGE AND STABILITY

Recommended storage for kit components is 2-8°C. All components are shipped and stored at 2-8°C. Reconstituted standards and controls can be frozen for future use but repeated freeze/thaw cycles should be avoided. Refer to expiration dates on all reagents prior to use. Do not mix reagents from different kits unless they have the same lot numbers.

Bioaffy 1000 nL CD can be stored at room temperature for up to 2 weeks after opening. Do not refrigerate CD after opening. Seal CD in original foil pouch with tape and protect from light.

REAGENT PRECAUTIONS

Sodium Azide or Proclin has been added to some reagents as a preservative. Although the concentrations are low, Sodium Azide and Proclin may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes and pipette tips: 10 µL-20 µL or 20 µL-100 µL
2. Reagent Reservoirs
3. Polypropylene Microfuge Tubes
4. Vortex Mixer
5. De-ionized water
6. Tween 20, Sigma Catalog # P7949 or equivalent.
7. Sodium Azide, Sigma Catalog # A3059 or equivalent
8. 10X PBS, Sigma Catalog # D5652-10X1L or equivalent
9. Absolute Alcohol (non-denatured), Fisher Catalog # BP2818-4 or equivalent
10. Microfuge
11. 0.22µm Stericup Filter, Millipore Cat # SCGVU11RE or equivalent

SAMPLE COLLECTION, STORAGE AND PREPARATION

A. Preparation of Serum Samples:

- Allow the blood to clot for at least 30 minutes before centrifugation for 10 minutes at 1,000xg. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple (>2) freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge for 5 minutes at 14,000xg prior to use in the assay to remove particulates.
- Serum samples are diluted 1:2 in Diluent GM3. Add 10 µL of sample to 10 µL of Diluent GM3 in polypropylene microfuge tubes and mix well.

B. Preparation of Plasma Samples:

- Plasma collection using EDTA as an anti-coagulant is recommended. Centrifuge for 10 minutes at 1,000xg within 30 minutes of blood collection. Remove plasma and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple (>2) freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge for 5 minutes at 14,000xg prior to use in the assay to remove particulates.
- Plasma samples are diluted 1:2 in Diluent GM3. Add 10 µL of sample to 10 µL of Diluent GM3 in polypropylene microfuge tubes and mix well.

NOTE:

- Avoid using samples with gross hemolysis or lipemia.
- All samples must be stored in polypropylene tubes. **DO NOT STORE SAMPLES IN GLASS.**

REAGENT PREPARATION

Serum Matrix Preparation

1. Use care in opening the lyophilized Serum Matrix vial. Using a pipette, reconstitute the Serum Matrix with 500 µL distilled or de-ionized water. Invert and mix gently, let sit for 5 minutes then mix well.

2. Transfer prepared matrix solution to a 1.5 mL microfuge tube and spin in a microfuge at 14,000xg for 5 minutes. Draw off the supernatant and transfer to a clean tube.

3. Dilute the above supernatant 1:2 with Diluent GM3 (e.g. combine 400 µL of the above supernatant with 400 µL of diluent Diluent GM3) and vortex to mix well.

Human Insulin Standard Preparation

1. Use care in opening the lyophilized Human Insulin Standard vial. Using a pipette, reconstitute the Standard with 100 µL distilled or de-ionized water. Invert and mix gently, let sit for 5 minutes then mix well.

2. Label 7 polypropylene microfuge tubes as Tubes 1-7. Add 90 µL of the above prepared Serum Matrix solution to tube 7. Add 10 µL of the reconstituted standard to tube 7 and mix well.

3. Add 60 µL of the above prepared Serum Matrix solution to each of the 6 tubes labeled 1-6. Prepare serial dilutions by adding 20 µL of the above prepared tube #7 standard to the #6 tube, mix well and transfer 20 µL of the #6 standard to the #5 tube, mix well and transfer 20 µL of the #5 standard to the #4 tube, mix well and transfer 20 µL of the #4 standard to the #3 tube, mix well and transfer 20 µL of the #3 standard to the #2 tube, mix well and transfer 20 µL of the #2 standard to the #1 tube and mix well. The 0 pg/mL standard (Background) will be the above prepared Serum Matrix solution.

   Note: Change tip for every dilution. Wet the tip with standard before dispensing. Unused portions of reconstituted standard should be stored in small aliquots at ≤ -20°C. Avoid multiple freeze/thaw cycles.

Tube #	Volume of Deionized Water to Add	Volume of Standard to Add	Standard Stock Concentration
Reconstituted standard (not used in assay)	100 µL	0	1 µg/mL

Tube #	Volume of prepared Matrix Serum Solution to Add	Volume of Standard to Add	Standard Concentration (pg/mL)
7	90 µL	10 µL of reconstituted standard	100,000
6	60 µL	20 µL of 7	25,000
5	60 µL	20 µL of 6	6,250
4	60 µL	20 µL of 5	1,563
3	60 µL	20 µL of 4	391
2	60 µL	20 µL of 3	97.7
1	60 µL	20 µL of 2	24.4

A. Human Insulin Quality Control Preparation

Use care in opening the lyophilized Quality Control vials. Reconstitute each Human Insulin Quality Control 1, Quality Control 2, and Quality Control 3 with 100 µL of distilled or de-ionized water and gently invert to ensure complete hydration. Label 3 polypropylene microfuge tubes as QC1, QC2, and QC3. Perform a 1:10 dilution of each Quality Control by adding 90 µL of the above prepared Serum Matrix solution to each tube. Add 10 µL of each Quality Control to the appropriate tube. Unused portions of the reconstituted Quality Controls should be stored in small aliquots at ≤ -20°C. Avoid further freeze/thaw cycles.

Tube #	Volume of prepared Serum Matrix Solution Add	Reconstituted Quality Control to Add	Final Concentration (pg/mL)
QC1	90 µL	10 µL of Reconstituted Quality Control 1	See tech sheet for range
QC2	90 µL	10 µL of Reconstituted Quality Control 2	See tech sheet for range
QC3	90 µL	10 µL of Reconstituted Quality Control 3	See tech sheet for range

REAGENT PREPARATION (continued)

B. Wash Buffer #1 – PBS-T (not provided)

PBS + 0.01% Tween is used as Wash Buffer #1. PBS-T should be prepared and filtered using a 0.22 µm filter as required in Gyros Gyrolab™ xP Workstation user manual for the running of all assays and used for Wash Station #1 and Wash Solution in the assay plate.

C. Wash Buffer #2 - pH 11 Wash Buffer

Reconstitute the lyophilized Wash Buffer with 1 liter of distilled or de-ionized water and stir on magnetic stir plate until all salts go into solution. Wash Buffer must then be filtered using a 0.22 µm filter. Store at room temperature for 1 week.

D. Preparation of Detection Antibody

Vortex Detection Antibody vial to mix well. Transfer all contents of Detection antibody vial to a clear microcentrifuge tube. Spin clear tube and contents down at 14,000xg for 5 minutes. Dilute the detection antibody 1:20 immediately after spinning by removing 8 µL of the detection antibody supernatant and adding to 152 µL of Detection Diluent Cat # GMDTDIL in the Mixing Bottle. Diluted detection must be mixed at time of use.

ASSAY PROCEDURE

1. Design Run on Gyrolab™ xP Workstation using the 3 step method **1000-3W-006-A** (method can be downloaded from Gyros SB website) including both Wash Station #1and #2 with the 1000 nL Bioaffy CD.

NOTE: Kit reagents are sufficient to run blank, standard curve, quality controls and 37 samples in duplicate on one Bioaffy 1000 nL CD.

2. Prepare all reagents and samples as instructed in this Human Insulin GyroMark HT protocol.

3. Fill in the standard concentrations listed in this protocol into the reagent list as prompted. Run all Standards, Blanks, Quality Controls, and Samples in duplicate. The Quality Controls can be listed as either Quality Controls or Samples in the assay set-up.

4. Generate a sample list for the number of samples being run (up to 37 when running in duplicate, or 40 if listing Quality Controls as Samples).

5. Execute Run on Gyrolab™ xP Workstation and acquire Loading List.

6. Add volume of Blank (Diluent GM3), prepared Standards, prepared Quality Controls 1, 2, and 3, and Diluted Samples to the appropriate wells of Reagent Plate #1 as instructed in the Loading List and cover with foil seal using foil seal adapter provided by Gyros AB with the Gyrolab™ xP Workstation.

7. Add volume of Wash Solution, Capture Antibody and prepared Detection Antibody to the appropriate wells of Reagent Plate #2 as instructed in the Loading List and cover with foil seal using foil seal adapter provided by Gyros AB with the Gyrolab™ xP Workstation.

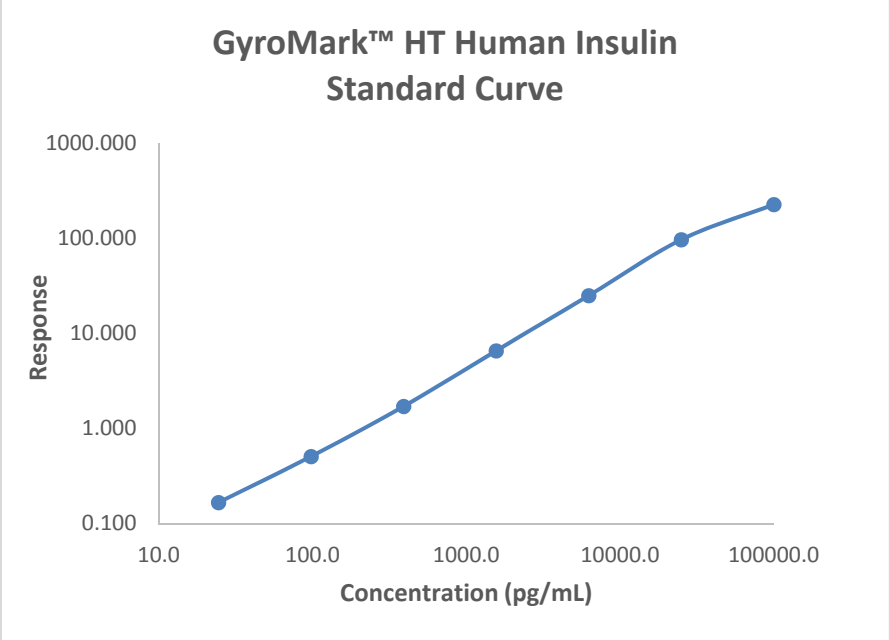
8. Ensure that sufficient Wash Buffer #1 and Wash Buffer #2 are connected to the Gyrolab™ xP Workstation.

9. Load Reagent Plate #1, Bioaffy 1000 nL CD, and Reagent Plate #2 as instructed in Run Execution.

10. Close hatch and execute run. (Prime if needed.)

11. When assay is complete, shut down server and evaluate using Gyrolab™ Evaluator.

GRAPH OF TYPICAL REFERENCE CURVE



Typical Standard Curve, not to be used to calculate data.

ASSAY CHARACTERISTICS

A. Sensitivity

The Minimum Detectable Concentration (MinDC) of Insulin is 14.3 pg/mL. It is calculated using MILLIPLEX® Analyst 5.1. It measures the true limits of detection for an assay by mathematically determining what the empirical MinDC would be if an infinite number of standard concentrations were run for the assay under the same conditions. This reported value is the mean plus 2 standard deviations of the MinDC of multiple assays (n= 8).

The **LLOQ** of Insulin is 24.4 pg/mL. It is the lowest concentration at which 20 replicates have CV of ≤ 20% and the percent recovery of the standard is still between 80%-120%.

The **ULOQ** of Insulin is 100,000 pg/mL. It is the highest concentration at which 20 replicates have CV of ≤ 20% and the percent recovery of the standard is still between 80%-120%.

ASSAY CHARACTERISTICS (continued)

B. Specificity

Human Proinsulin ND
Human C-Peptide ND

ND = Non-detectable

C. Precision

Intra-Assay Variation

Sample	Mean Insulin Levels (pg/mL)	Intra-Assay %CV
1	355	<10
2	474	<10
3	1764	<10
4	9633	<10

Inter-Assay Variation

Sample	Mean Insulin Levels (pg/mL)	Inter-Assay %CV
1	411	<15
2	1988	<15
3	9605	<15

The assay variation of EMD Millipore's Human Insulin GyroMark™ HT kit was studied on samples at various levels on the Insulin standard curve. The mean intra-assay variation was calculated from results of sixteen determinations of the indicated samples (n=4). The mean inter-assay variation of each sample (n = 3) was calculated from results of 8 separate assays with duplicate samples in each assay.

ASSAY CHARACTERISTICS (continued)

D. Spike Recovery of Insulin in Human Serum and Plasma

Sample	Insulin Added (pg/mL)	Expected (pg/mL)	Observed (pg/mL)	Recovery %
Serum 1	Neat	65	65	
	98	162	189	117
	391	455	521	114
	1563	1627	1876	115
	6250	6315	6774	107
	25000	25065	26659	106
Serum 2	Neat	87	87	
	98	184	179	97
	391	477	445	93
	1563	1649	1452	88
	6250	6337	5098	80
	25000	25087	18333	73
Serum 3	Neat	64	64	
	98	161	170	105
	391	454	468	103
	1563	1626	1717	106
	6250	6314	6531	103
	25000	25064	22936	92
Plasma 1	Neat	75	75	
	98	172	175	101
	391	465	501	108
	1563	1637	1675	102
	6250	6325	6214	98
	25000	25075	22630	90
Plasma 2	Neat	126	126	
	98	224	251	112
	391	517	575	111
	1563	1689	1894	112
	6250	6376	6525	102
	25000	25126	22654	90
Plasma 3	Neat	118	118	
	98	215	205	95
	391	508	538	106
	1563	1680	1825	109
	6250	6368	6516	102
	25000	25118	24026	96
			Average	101

Varying amounts of Insulin were added to individual serum and plasma samples (n=6) and the resulting Insulin content of each sample was assayed using the Human Insulin GyroMark™ HT kit. The recovery equals [(observed Insulin / (spiked Insulin concentration + basal Insulin level)] x 100%.

ASSAY CHARACTERISTICS (continued)

E. Linearity of Insulin Human Serum and Plasma Sample Dilution

Sample	Dilution factor	Expected (pg/mL)	Observed (pg/mL)	Expected %
Serum 1	Neat	594	594	
	2	297	261	88
	4	148	148	100
	8	74	76	102
	16	37	37	100
	32	19	ND	
Serum 2	Neat	475	475	
	2	237	229	97
	4	119	116	97
	8	59	53	89
	16	30	ND	
	32	15	ND	
Serum 3	Neat	1132	1132	
	2	566	537	95
	4	283	272	96
	8	141	135	95
	16	71	66	93
	32	35	32	92
Plasma 1	Neat	953	953	
	2	477	480	101
	4	238	243	102
	8	119	115	97
	16	60	57	95
	32	30	27	90
Plasma 2	Neat	596	596	
	2	298	341	114
	4	149	186	125
	8	74	87	117
	16	37	46	125
	32	19	ND	
Plasma 3	Neat	202	202	
	2	101	95	94
	4	51	55	109
	8	25	ND	
	16	13	ND	
	32	6	ND	
			Average	101

Insulin human serum and plasma samples (n=6) with the indicated Insulin concentrations were assayed. The resulting dilution factors of neat (1:2 diluted samples treated as neat), 2, 4, 8, 16 and 32 were applied in the calculation of observed Insulin concentrations. % expected equals (observed/expected) x 100%.
ND = Non-detectable

QUALITY CONTROLS

The ranges for Quality Control 1, 2, and 3 are provided on the card insert or can be located at the EMD Millipore website www.millipore.com/techlibrary/index.do

TROUBLESHOOTING GUIDE

1. To obtain reliable and reproducible results the operator should carefully read this manual and fully understand how to design and execute a run on the Gyros Gyrolab™ xP Workstation as outlined in the Gyros Gyrolab™ xP Workstation User Guide before attempting to run the assay.
2. Follow the recommended maintenance schedule outlined in the Gyrolab™ User Guide. It is recommended that the needles be sanitized with Needle Sanitize Solution (3% sodium hypochlorite) weekly when biological samples are used.
3. Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
4. Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started all steps should be completed with precise timing and without interruption.
5. Avoid cross contamination of any reagents or samples to be used in the assay.
6. Make sure all reagents and samples are added to the bottom of each well of the microtiter plates and no bubbles are present in the wells.
7. Careful and complete mixing of solutions is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.

TROUBLESHOOTING GUIDE (continued)

Issue	Possible Cause	Action
No response values generated	CD is upside down	Make sure text on CD is readable
	Samples and Reagents not loaded in proper wells of microplate as instructed in loading list	Load according to loading list
	Foil seal not applied correctly	Use foil seal adapter to seal properly aligned
	Sample and Reagent plates not loaded in proper order	Load Sample microplate first as instructed and reagent plate second as instructed by Gyrolab™ prompts
Instrument errors generated	Insufficient volume of samples or reagents loaded	Load sample and reagent volumes according to loading list
	Bubbles in microplate samples or reagents	Remove air bubbles from samples and reagents before sealing microplate. Remove air bubble using pipet and ensure proper amount of sample/reagent remains or seal microplate and spin plates at 3000xg for 2 minutes
	Bubbles in the syringe pumps	Ensure degasser is on and working. Prime Gyrolab™ if bubbles are present in pump syringes. If bubbles are still present refer to section E1.7.1 of Gyrolab™ User Guide or call Gyros service technician
Low response	Standards and samples not stored properly	Samples should be thawed on ice and standards handled/stored according to assay protocol
	Detection Antibody not freshly prepared/improperly prepared	Detection Antibody must be prepared at time of use following instructions in assay protocol
High CVs	Did not centrifuge the detection antibody before use	Spin the detection antibody at 14,000xg for 5 minutes before diluting to final concetration and then vortex final dilution well
	Reagents not stored as recommended in protocol	All reagents must be prepared and stored according to assay protocol
	Insufficient wash solution connected to the machine	Ensure sufficient volumes of wash solutions are prepared and connected
	Insufficient samples/reagents loaded in microtiter plate	Ensure samples/reagents volumes are loaded into microtiter plates as instructed in loading list
	Air bubbles are present in microtiter plate or pumps	Ensure no air is present in micrototer plate - remove if needed. Prime machine if air is observed in pumps
	Did not use Wash Buffer #2 to prevent carryover	Prepare and use Wash Buffer #2 as instructed in assay protocol
	Spinner/Needles contaminated	Clean surfaces and perform needle desorb according to Gyrolab User Guide
High background	Detection antibody not prepared properly	Prepare detection antibody according to assay protocol
	Insufficent wash buffer added to microtiter plate	Ensure wash buffer is added to microtiter plate as instructed in the loading list
	Did not use Wash Buffer #2 to prevent carryover	Prepare and use Wash Buffer #2 as instructed in assay protocol
	Spinner/Needles contaminated	Clean surfaces and perform needle desorb according to Gyrolab™ User Guide

REPLACEMENT REAGENTS

Reagents	Cat. #
Bioaffy 1000 nL CD	P0004253
Human Insulin Standard Human Insulin Quality Controls 1, 2 and 3 Human Insulin Capture Antibody Human Insulin Detection Antibody Serum Matrix	SK-GYHINS
Diluent GM3	GMDIL-3
Detection Diluent	GMDTDIL
pH 11 Wash Buffer	P0020087
Mixing Bottle	----------
Sample Plates/Foil Seals	GM-PLATE

ORDERING INFORMATION

To place an order:

To assure the clarity of your custom kit order, please FAX the following information to our customer service department:

Include:

- Your name, telephone and/or fax number
- Customer account number
- Shipping and billing address
- Purchase order number
- Catalog number and description of product
- Quantity of kits

FAX: (636) 441-8050

Toll-Free US: 1-800-MILLIPORE
781-533-8870

Mail Orders: EMD Millipore Corporation
6 Research Park Drive
St. Charles, Missouri 63304 U.S.A.

For European Customers:

To best serve our European customers in placing an order or obtaining additional information about GyroMark™ HT Kit products, please contact your multiplex specialist or sales representative or email our European Customer Service at:

Austria	AUCustomerService@merckgroup.com
Belgium	BENLCustomerService@merckgroup.com
Denmark	denmark@merckgroup.com
France	FRCustomerService@merckgroup.com
Finland	Asiakaspalvelu@merckgroup.com
Germany	GECustomerService@merckgroup.com
Ireland	IECustomerService@merckgroup.com
Italy	CSR-IT@merckgroup.com
Netherlands	BENLCustomerService@merckgroup.com
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Sweden	Kundservice@merckgroup.com
Switzerland	SZCustomerService@merckgroup.com
UK	UKCustomerService@merckgroup.com

ORDERING INFORMATION (continued)

Conditions of Sale

For Research Use Only. Not for Use in Diagnostic Procedures.

Material Safety Data Sheets (MSDS)

Material Safety Data Sheets for EMD Millipore products may be ordered by fax or phone or through our website at www.emdmillipore.com/techlibrary/index.do.

Technical Services

http://www.emdmillipore.com/techservices
To contact by phone
For North America: Toll-Free US: 1-(800) 221-1975 or 1-(781) 533-8045
Outside North America, contact your local office
http://www.emdmillipore.com/offices