Human Resistin

96-Well Plate

Cat. # EZHR-95K

HUMAN RESISTIN ELISA KIT
96-Well Plate (Cat. # EZHR-95K)

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I INTENDED USE

This Human Resistin ELISA kit is used for the non-radioactive quantification of human resistin in serum, plasma and other biological media. One kit is sufficient to measure 38 unknown samples in duplicate. ***This kit is for research purposes only.***

II. PRINCIPLES OF PROCEDURE

This assay is based, sequentially, on: 1) capture of human resistin from sample by a monoclonal antibody, immobilized in the wells of a microwell plate, 2) washing off unbound materials including free materials from samples, 3) binding of the biotinylated monoclonal human resistin antibody to the other side of captured human resistin molecules, 4) conjugation of SA-HRP (Poly-HRP-labeled streptavidin) enzyme to the biotinylated antibodies, and 5) quantification of bound detection conjugate by monitoring SA-HRP enzyme activity in the presence of TMB (tetramethylbenzidine) substrates. The enzyme activity is measured spectrophotometrically by the absorbency at 450 nm due to production of the photometric product. Since the amount of photometric product is directly proportional to the concentration of human resistin in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of human resistin.

III. REAGENTS SUPPLIED

Each kit is sufficient to run one 96-well microtiter plate and contains the following reagents:

A. Human Resistin ELISA Plate
Coated with anti- Human Resistin Monoclonal Antibody
Quantity: 1 plate
Preparation: Ready to use

B. Adhesive Plate Sealer
Quantity: 2 Sheets
Preparation: Ready to use

C. 10X HRP Wash Buffer Concentrate
10X concentrate of 50 mM TBS Buffer containing 0.05%Tween 20
Quantity: 2 bottles containing 50 mL each
Preparation: Dilute 1:10 with distilled or deionized water

D. Human Resistin Standards
Human Resistin, lyophilized.
Quantity: 2.5 ng/0.25 mL (10 ng/mL) upon hydration.
Preparation: Contents Lyophilized. Reconstitute with 250 µL distilled or deionized water to obtain 10.0 ng/mL.

III. REAGENTS SUPPLIED (continued)

E. Human Resistin Quality Controls 1 and 2

One vial each, lyophilized, containing Human Resistin in Assay Buffer.
Quantity: 0.25 mL/vial upon hydration.
Preparation: Contents Lyophilized. Reconstitute each vial with 250 µL distilled or deionized water.

F. Assay Buffer

0.05M PBS, pH 7.4, containing 0.025 M EDTA, 0.08% Sodium Azide and 1% BSA.
Quantity: 12 mL
Preparation: Ready to use

G. Human Resistin Detection Antibody

Biotinylated anti-human resistin monoclonal antibody
Quantity: 9 mL
Preparation: Ready to use

H. Enzyme Solution

Pre-titered Streptavidin-Horseradish Peroxidase Conjugate (SA-HRP)
Quantity: 12 mL
Preparation: Ready to use

I. Substrate (Light sensitive, avoid unnecessary exposure to light)

3, 3', 5, 5'-tetramethylbenzidine (TMB)
Quantity: 12 mL
Preparation: Ready to use

J. Stop Solution (Caution: Corrosive Solution)

Quantity: 12 mL
Preparation: Ready to use

IV. STORAGE AND STABILITY

Prior to use, all components in the kit can be stored up to 2 weeks at 2-8°C. For longer storage (> 2 weeks), freeze diluted Wash Buffer, Assay Buffer, Standards, Controls and reconstituted Standards and Controls at ≤ –20°C. Minimize repeated freeze and thaw of the Standards and Quality Controls. Refer to expiration dates on all reagents prior to use. Do not mix reagents from different kits unless they have the same lot numbers.

V. REAGENT PRECAUTIONS

A. Sodium Azide

Sodium Azide has been added to reagents as a preservative at a concentration of 0.08%. Although it is at a minimum concentration, Sodium Azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with large volume of water to prevent azide build up.

B. Hydrochloric Acid

Hydrochloric Acid is corrosive and can cause eye and skin burns. It is harmful if swallowed and can cause respiratory and digestive tract burns. Avoid contact with skin and eyes.

VI. MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipette with tips, 10µL - 200µL
2. Multi-channel pipette, 50µL - 300µL
3. Reagent reservoirs
4. Vortex mixer
5. Refrigerator
6. Deionized water
7. Microtiter plate reader capable of reading absorbency at 450 nm
8. Microtiter plate shaker
9. Absorbent Paper or Cloth

VII. SAMPLE COLLECTION AND STORAGE

1. To prepare serum samples, whole blood is directly drawn into a centrifuge tube that contains no anti-coagulant. Let blood clot at room temperature for 30 minutes. Promptly centrifuge the clotted blood at 2,000 to 3,000 x g for 15 minutes at 4 ± 2°C. Transfer and store serum samples in separate tubes. Date and identify each sample. Use freshly prepared serum or aliquot and store samples at ≤ –20°C for later use. For long-term storage, keep at -70 °C. Avoid freeze/thaw cycles.

2. To prepare plasma samples, whole blood should be collected into centrifuge tubes containing enough K_3EDTA to achieve a final concentration of 1.735 mg/mL and centrifuged immediately after collection. Observe the same precautions in the preparation of serum samples.

3. If heparin is to be used as an anticoagulant, the effect on the assay outcome at the dose of heparin used should be pre-determined.

4. Avoid using samples with gross hemolysis or lipemia.

VIII. SAMPLE PREPARATION

1. Allow all the reagents to come to room temperature.
2. Dilute serum or plasma samples 1:10 in Assay Buffer. Cellular extract and culture media dilutions will vary.

IX. STANDARD AND QUALITY CONTROLS PREPARATION

Human Resistin Standard Preparation

1. Use care in opening the lyophilized Standard vial. Using a pipette, reconstitute the Human Resistin Standard with 250 µL distilled or deionized water into the glass vial to give a 10 ng/mL concentration of Standard. Invert and mix gently, let sit for 5 minutes then vortex gently.
2. Label six tubes 5, 2.5, 1.25, 0.625, 0.312, and 0.16 ng/mL. Add 100 µL Assay Buffer to each of the six tubes. Prepare serial dilutions by adding 100 µL of the 10 ng/mL reconstituted standard to the 5 ng/mL tube, mix well and transfer 100 µL of the 5 ng/mL reconstituted standard to the 2.5 ng/mL tube, mix well and transfer 100 µL of the 2.5 ng/mL Standard to the 1.25 ng/mL tube, mix well and transfer 100 µL of the 1.25 ng/mL Standard to the 0.625 ng/mL tube, mix well and transfer 100 µL of the 0.625 ng/mL Standard to the 0.312 ng/mL tube, mix well and transfer 100 µL of the 0.312 ng/mL Standard to the 0.16 ng/mL tube and mix well.

Standard Concentration ng/mL	Volume of Deionized Water to Add	Volume of Standard to Add
10	250 µL	0

Standard Concentration ng/mL	Volume of Assay Buffer to Add	Volume of Standard to Add
5	100 µL	100 µL of 10 ng/mL
2.5	100 µL	100 µL of 5 ng/mL
1.25	100 µL	100 µL of 2.5 ng/mL
0.625	100 µL	100 µL of 1.25 ng/mL
0.312	100 µL	100 µL of 0.625 ng/mL
0.16	100 µL	100 µL of 0.312 ng/mL

Human Resistin Quality Control 1 and 2 Preparation

Use care in opening the lyophilized Quality Control vials. Using a pipette, reconstitute each of the Human Resistin Quality Control 1 and Quality Control 2 with 250 µL distilled or deionized water into the glass vials. Invert and mix gently, let sit for 5 minutes then mix well.

Note: Do not use a Repeater pipette. Change tip for every dilution. Wet tip with Standard before dispensing. Unused portions of Standard and Quality Controls should be aliquoted and stored at ≤ -20°C immediately. Avoid multiple freeze/thaw cycles.

X. ASSAY PROCEDURE

Pre-warm all reagents to room temperature prior to setting up the assay.

1. Dilute the 10X concentrated Wash Buffer 10 fold by mixing the entire content of each bottle of Wash Buffer with 450 mL deionized or distilled water (Dilute both bottles with 900 mL deionized water).

2. Remove the Microtiter Assay Plate from the foil pouch and add 300 µL of diluted Wash Buffer to each well. Incubate at room temperature for 5 minutes. Decant Wash Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times. **Do not let wells dry before proceeding to the next step. If an automated machine is used for the assay, follow the manufacturer's instructions for all washing steps described in this protocol.**

3. Add 60 µL Assay Buffer into all wells.

4. Add in duplicate 20 µL of Assay Buffer to blank wells.

5. Add in duplicate 20 µL of Human Resistin Standards in order of ascending concentration to the appropriate wells. Add in duplicate 20 µL QC1 and 20 µL QC2 to the appropriate wells. Add sequentially 20 µL of diluted serum or plasma samples in duplicate to the remaining wells.

6. Cover the plate with plate sealer and incubate at room temperature for 1.5 hours on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400 to 500 rpm.

7. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in the wells.

8. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.

9. Add 80 µL Detection Antibody to all wells. Cover the plate with plate sealer and incubate at room temperature for 1 hour on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400 to 500 rpm.

10. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in the wells.

11. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.

X. ASSAY PROCEDURE (continued)

12. Add 80 µL Enzyme Solution to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 30 minutes on the microtiter plate shaker.

13. Remove sealer, decant solutions from the plate, and tap plate to remove the residual fluid.

14. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.

15. Add 80 µL of Substrate Solution to each well, cover plate with sealer and shake on the plate shaker for **approximately** 5 to 20 minutes. Blue color should be formed in wells of Resistin standards with intensity proportional to increasing concentrations of Resistin.

NOTE: Please be aware that the blue color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please visually monitor the color development to optimize the incubation time.

16. Remove sealer and add 80 µL Stop Solution **[CAUTION: CORROSIVE SOLUTION]** and shake plate by hand to ensure complete mixing of solution in all wells. The blue color should turn to yellow after acidification. Read absorbance at 450 nm and 590nm in a plate reader within 5 minutes and ensure that there are no air bubbles in any well. Record the difference of absorbance units. The absorbance of highest Resistin standard should be approximately 2.5-3.2, or not to exceed the capability of the plate reader used.

Assay Procedure for Human Resistin ELISA kit (Cat. # EZHR-95K)

	Step 1	Step 2	Step 3-4	Step 5	Step 6-8	Step 9	Step 9-11	Step 12	Step 12-14	Step 15	Step 15	Step 16	Step 16
Well #	Dilute each bottle 10X Wash Buffer with 450mL Deionized Water.	Wash plate 1X with 300 µL Wash Buffer. Incubate 5 minutes at Room Temperature. Remove residual buffer by tapping smartly on absorbent towels	Assay Buffer	Standards/ Controls/ Samples	Seal, Agitate, Incubate 1.5 hours at Room Temperature. Wash 3X with 300 µL Wash Buffer	Detection Ab	Seal, Agitate, Incubate 1 hour at Room Temperature. Wash 3X with 300 µL Wash Buffer	Enzyme Solution	Seal, Agitate, Incubate 30 minutes at Room Temperature. Wash 3X with 300 µL Wash Buffer	Substrate	Seal, Agitate, Incubate 5 - 20 minutes at Room Temperature.	Stop Solution	Read Absorbance at 450 nm and 590 nm.
A1, B1			80 µL			80 µL		80 µL		80 µL		80 µL	
C1, D1			60 µL	20 µL of 0.16 ng/mL Standard									
E1, F1			60 µL	20 µL of 0.312 ng/mL Standard									
G1, H1			60 µL	20 µL of 0.625 ng/mL Standard									
A2, B2			60 µL	20 µL of 1.25 ng/mL Standard									
C2, D2			60 µL	20 µL of 2.5 ng/mL Standard									
E2, F2			60 µL	20 µL of 5 ng/mL Standard									
G2, H2			60 µL	20 µL of 10 ng/mL Standard									
A3, B3			60 µL	20 µL of QC I									
C3, D3			60 µL	20 µL of QC II									
E3, F3			60 µL	20 µL of Diluted Sample									
G3, H3			60 µL	20 µL of Diluted Sample									
A4, B4 ↓			60 µL	20 µL of Diluted Sample									

XI. MICROTITER PLATE ARRANGEMENT

Human Resistin ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	1.25 ng/mL	QC 1									
B	Blank	1.25 ng/mL	QC 1									
C	0.16 ng/mL	2.5 ng/mL	QC 2									
D	0.16 ng/mL	2.5 ng/mL	QC 2									
E	0.312 ng/mL	5 ng/mL	Sample									
F	0.312 ng/mL	5 ng/mL	Sample									
G	0.625 ng/mL	10 ng/mL	Sample									
H	0.625 ng/mL	10 ng/mL	Sample									

XII. CALCULATIONS

The dose-response curve of this assay fits best to a sigmoidal 5-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 5-parameter logistic function.

It is important to make an appropriate mathematical adjustment to accommodate for the dilution factor.

XIII. ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of Human Resistin that can be detected by this assay is 0.16 ng/mL.

B. Precision

Within Assay Variation

Sample No.	Mean Resistin Levels, ng/mL	Within %CV
1	64.1	4.0
2	12.9	3.2
3	20.2	7.0
4	10.2	3.8

Between Assay Variation

Sample No.	Mean Resistin Levels, ng/mL	Between %CV
1	0.67	7.7
2	3.73	7.1

The assay variations of Millipore Human Resistin ELISA Kits were studied on human serum samples with varying concentrations of resistin. The mean within variation was calculated from results of ten duplicate determinations in each assay of the indicated samples. The mean between variations of each sample was calculated from results of three separate assays with duplicate samples in each assay.

XIII. ASSAY CHARACTERISTICS (continued)

C. Recovery

Spike & Recovery of Resistin in Human Serum

Sample #	Resistin added ng/mL	Expected ng/mL	Observed ng/mL	% of Recovery
1	0	1.17	1.17	100
	0.2	1.37	1.36	99.3
	1.0	2.17	1.96	90.3
	5.0	6.17	5.60	90.8
2	0	0.68	0.68	100
	0.2	0.88	0.91	103
	1.0	1.68	1.54	91.7
	5.0	5.68	5.30	93.3
3	0	1.49	1.49	100
	0.2	1.69	1.68	99.4
	1.0	2.49	2.46	98.8
	5.0	6.49	5.98	92.1
4	0	0.69	0.69	100
	0.2	0.89	0.96	108
	1.0	1.69	1.68	99.4
	5.0	5.69	5.31	93.3

Varying concentrations of human resistin were added to four human serum samples and the resistin content was determined by four separate assays. Percent recovery = observed Resistin concentrations ÷ expected Resistin concentrations x 100%.

XIII. ASSAY CHARACTERISTICS (continued)

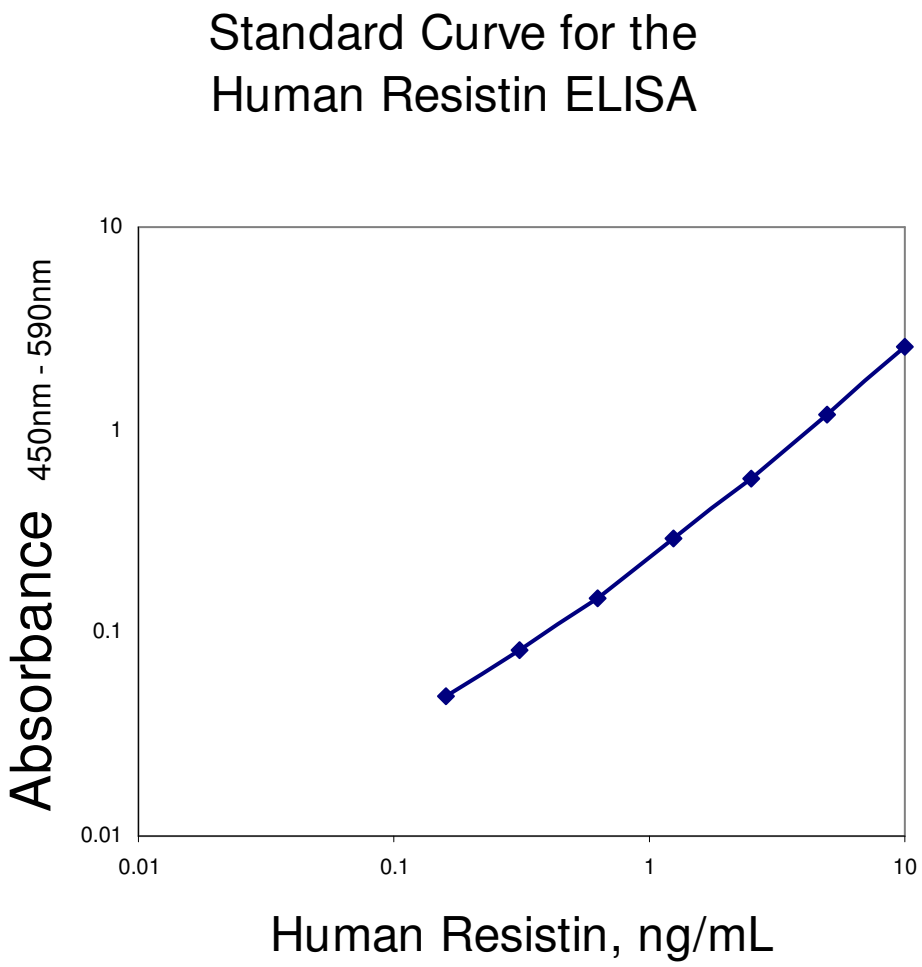
D. Linearity

Effect of Serum Dilution

Sample No.	Sample Dilution	Expected ng/mL	Observed ng/mL	% Of Expected
1	0	2.53	2.53	100
	2	1.16	1.27	109
	4	0.60	0.64	107
	8	0.32	0.32	100
2	0	1.49	1.49	100
	2	0.75	0.63	84
	4	0.37	0.31	84
	8	0.19	0.17	89
3	0	2.04	2.04	100
	2	1.02	0.86	84
	4	0.51	0.48	94
	8	0.26	0.22	85

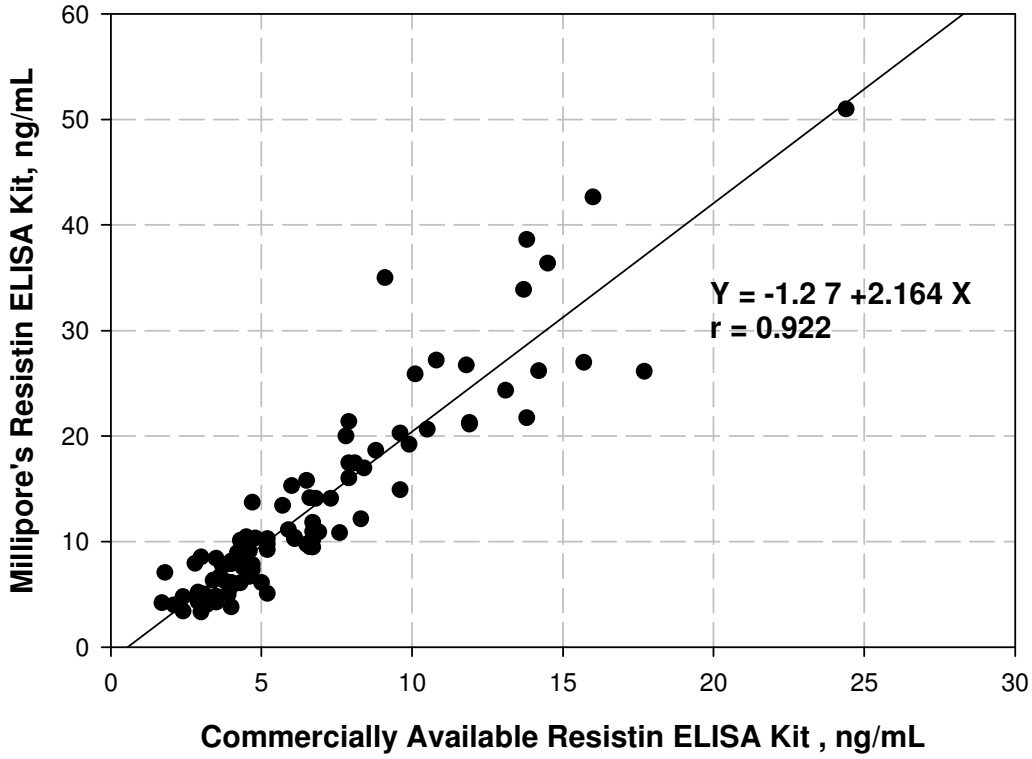
Three human serum samples with the indicated sample volumes were assayed in three separate experiments. The samples were diluted for 10-fold following the assay protocol. Further dilutions were made and analyzed. The mean resistin levels and percent of expected from four duplicates determinations are shown. % expected = observed/expected x 100%.

XIV. HUMAN RESISTIN STANDARD CURVE



XV. METHOD COMPARISON

Millipore's Human Resistin ELISA Kit
vs. a commercially available
Human Resistin ELISA Kit



XVI. QUALITY CONTROLS

The ranges for Quality Control 1 and 2 are provided on the card insert or can be located at the Millipore website www.millipore.com

XVII. TROUBLESHOOTING GUIDE

1. To obtain reliable and reproducible results the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
2. Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
3. 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.
4. Avoid cross contamination of any reagents or samples to be used in the assay.
5. Make sure all reagents and samples are added to the bottom of each well.
6. Careful and complete mixing of solutions in the well is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
7. Remove any air bubble formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
8. Do not let the absorbency reading of the highest standard reach 3.0 units or higher after acidification.
9. High absorbance in background or blank wells could be due to 1) cross well contamination by standard solution or sample or 2) inadequate washing of wells with Wash Buffer or 3) overexposure to light after substrate has been added.

XVIII. REFERENCES

1. Tijsen P. "Practice and Theory of Enzyme Immunoassays" in Burdon RH and Knippenberg PH (Ed.), Laboratory Techniques in Biochemisrty and Molecular Biology. Amsterdam/NY: Elsevier, 1985

2. Steppan GM et al., *2001. Nature* 409(18):307.

3. Kim KH et al., *2001. J. Biol. Chem. 276(14):* 11252.

4. Way JM et al., *2001. J. Biol. Chem.* 276(28):25651.

5. Smith U and Nagaev I. *2001. Biochem. Biophy. Res. Comm.* 285 **:** 561.

6. Moore GB et al., *2001. Biochem. Biophy. Res. Comm.* 286(4):735.

XIX. REPLACEMENT REAGENTS

Reagents	**Cat. #**
Human Resistin ELISA Plate	EP95
10X HRP Wash Buffer Concentrate	EWB-HRP
Human Resistin Standards	E8095-K
Human Resistin Quality Controls 1 and 2	E6095
Assay Buffer	EABIR
Human Resistin Detection Antibody	E1095
Enzyme Solution	EHRP-3
Substrate	ESS-TMB3
Stop Solution	ET-TMB

XIX. ORDERING INFORMATION

A. To place an order:

For USA Customers:

Please provide the following information to our customer service department to expedite your telephone, fax or mail order:

1. Your name, telephone and/or fax number
2. Customer account number
3. Shipping and billing address
4. Purchase order number
5. Catalog number and description of product
6. Quantity and product size

TELEPHONE ORDERS:
Toll Free US (800) MILLIPORE

FAX ORDERS: (636) 441-8050
MAIL ORDERS: Millipore
6 Research Park Drive
St. Charles, Missouri 63304 U.S.A.

For International Customers:

To best serve our international customers, it is Millipore's policy to sell our products through a network of distributors. To place an order or to obtain additional information about Millipore products, please contact your local distributor.

B. Conditions of Sale

All products are for research or manufacturing use only. They are not intended for use in clinical diagnosis or for administration to human or animals. All products are intended for *in vitro* use only.

C. Material Safety Data Sheets (MSDS)

Material safety data sheets for Millipore products may be ordered by fax or phone. See Section A above for details on ordering.