

Rat FGF-23 ELISA

96-Well Plate

Cat. # EZRFGF23-42K

Rat FGF-23 ELISA KIT
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RAT FGF-23 ELISA KIT
96-Well Plate (Cat. # EZRFGF23-42K)

I. INTENDED USE

This Rat FGF-23 ELISA kit is used for the non-radioactive quantification of Rat FGF-23 in serum, plasma, and tissue culture samples. This kit specifically measures native Rat FGF-23. One kit is sufficient to measure 41 unknown samples in duplicate. ***This kit is for Research Use Only. Not for Use in Diagnostic Procedures.***

II. PRINCIPLES OF PROCEDURE

This assay is a Sandwich ELISA based, sequentially, on: 1) concurrent capture of Rat FGF-23 molecules from samples to the wells of a microtiter plate coated with a polyclonal rabbit anti- FGF-23 antibody, and binding of a second biotinylated polyclonal goat anti-FGF-23 antibody to the captured molecules , 2) washing of unbound materials from samples, 3) binding of streptavidin-horseradish peroxidase conjugate to the immobilized biotinylated antibodies, 4) washing of excess of free enzyme conjugates, and 5) quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3',5,5'-tetramethylbenzidine. The enzyme activity is measured spectrophotometrically by the increased absorbance at 450 nm – 590 nm after acidification of formed products. Since the increase in absorbance is directly proportional to the amount of captured Rat FGF-23 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 Rat FGF-23.

III. REAGENTS SUPPLIED

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

A. FGF-23 ELISA Plate

Coated with Rabbit anti- FGF-23 Antibodies

Quantity: 1 Strip Plate

Preparation: Ready to Use

Note: Unused strips should be resealed in the foil pouch with the desiccant provided .

B. Adhesive Plate Sealer

Quantity: 2 sheets

Preparation: Ready to Use

C. 10X HRP Wash Buffer Concentrate

10X concentrate of 50 mM Tris Buffered Saline containing Tween 20

Quantity: 2 bottles containing 50 mL each

Preparation: Dilute 1:10 with distilled or deionized water

D. FGF-23 Standard

Purified Recombinant FGF-23, lyophilized.

Quantity: 0.25 mL upon hydration

Preparation: Reconstitute with 0.25 mL distilled or deionized water. See insert for concentration.

E. FGF-23 Quality Controls 1 and 2

One vial each, lyophilized, containing purified recombinant FGF-23 at two different levels.

Quantity: 0.25 mL/bottle upon hydration

Preparation: Reconstitute each vial with 0.25 mL distilled or deionized water.

F. Matrix Solution

Quantity: 1.5 mL, bottle

Preparation: Dilute bottle with 1.5 mL Assay Buffer and mix well prior to use.

G. Assay Buffer

0.05M PBS, pH 6.8, containing proprietary protease inhibitors, with Tween 20, 0.08% Sodium Azide and 1% BSA.

Quantity: 12 mL

Preparation: Ready to Use

H. FGF-23 Detection Antibody

Pre-titered Biotinylated Goat anti- FGF-23 Antibody

Quantity: 12 mL

Preparation: Ready to Use

III. REAGENTS SUPPLIED (continued)

I. Enzyme Solution

Pre-titered Streptavidin-Horseradish Peroxidase Conjugate in Buffer

Quantity: 12 mL

Preparation: Ready to Use

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

3, 3', 5, 5'-tetramethylbenzidine in buffer

Quantity: 12 mL

Preparation: Ready to Use.

K. Stop Solution (Caution: Corrosive Solution)

0.3 M HCl

Quantity: 12 mL

Preparation: Ready to Use

IV. 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 thaws 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.

V. REAGENT PRECAUTIONS

A. Sodium Azide

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.

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. Do not swallow or ingest.

See next page for Full Hazardous Labels for components of this kit.

Full Hazardous Component Labels:

Ingredient, Cat #		Full Label	
Rat Fibroblast Growth Factor-23 Quality Controls 1 & 2	E6042-K	 	<p>Warning. Harmful if swallowed. Toxic to aquatic life with long lasting effects. Avoid release to the environment.</p>
Rat Fibroblast Growth Factor-23 Standard	E8042-K	 	<p>Warning. Harmful if swallowed. Toxic to aquatic life with long lasting effects. Avoid release to the environment.</p>
Stop Solution	ET-TMB		<p>Warning. May be corrosive to metals.</p>
10X HRP Wash Buffer Concentrate	EWB-HRP		<p>Warning. May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.</p>

VI. MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes and Pipette Tips: 10 μ L - 20 μ L or 20 μ L - 100 μ L
2. Multi-Channel Pipettes and Pipette Tips: 5 μ L ~ 50 μ L and 50 μ L ~ 300 μ L
3. Buffer and Reagent Reservoirs
4. Vortex Mixer
5. Distilled or Deionized Water
6. Microtiter Plate Reader capable of reading absorbency at 450 nm
7. Orbital Microtiter Plate Shaker
8. 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 min.
2. Promptly centrifuge the clotted blood at 2,000 to 3,000 x g for 15 minutes at 2-8°C.
3. Transfer and store serum samples in separate tubes. Date and identify each sample.
4. Use freshly prepared serum or aliquot and store samples at $\leq -20^{\circ}\text{C}$ for later use. For long-term storage, keep at -70°C . Avoid multiple freeze/thaw cycles (>2).
5. 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.
6. 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.
7. Avoid using samples with gross hemolysis or lipemia.

VIII. REAGENT PREPARATION

A. FGF-23 Standard Preparation

1. Use care in opening the lyophilized Standard vial. Using a pipette, reconstitute the FGF-23 Standard with 0.25 mL distilled or deionized water to give a concentration described on the analysis sheet included in the kit. Invert and mix gently, let sit for 5 minutes then vortex gently.
2. Label five tubes 1, 2, 3, 4, and 5. Add 0.150 mL Assay Buffer to each of the five tubes. Prepare serial dilutions by adding 0.075 mL of the reconstituted standard to Tube 1, mix well and transfer 0.075 mL of Tube 1 to Tube 2, mix well and transfer 0.075 mL of Tube 2 to Tube 3, mix well and transfer 0.075 mL of Tube 3 to Tube 4, mix well and transfer 0.075 mL of Tube 4 to Tube 5, mix well.

Note: Do not use a Repeater pipette. Change tip for every dilution. Wet tip with Standard before dispensing. Unused portions of reconstituted standard should be stored at $\leq -20^{\circ}\text{C}$. Avoid multiple freeze/thaw cycles (>2).

Volume of Deionized Water to Add	Volume of Standard to Add	Standard Concentration (ng/mL)
0.25 mL	0	X (refer to analysis sheet for exact concentration)

Tube #	Volume of Assay Buffer to Add	Volume of Standard to Add	Standard Concentration (ng/mL)
Tube 1	0.150 mL	0.075 mL of Reconstituted Standard	X/3
Tube 2	0.150mL	0.075 mL of Tube 1	X/9
Tube 3	0.150 mL	0.075 mL of Tube 2	X/27
Tube 4	0.150 mL	0.075 mL of Tube 3	X/81
Tube 5	0.150 mL	0.075 mL of Tube 4	X/243

B. FGF-23 Quality Control 1 and 2 Preparation

Use care in opening the lyophilized Quality Control vials. Using a pipette, reconstitute each of the FGF-23 Quality Control 1 and Quality Control 2 with 0.25 mL distilled or deionized water into the vials. Invert and mix gently, let sit for 5 minutes then mix well.

C. Matrix Solution Preparation

Dilute Matrix Solution with 1.5 mL of Assay Buffer and mix well. **(Do not use matrix if testing TISSUE CULTURE samples –substitute with 50 μL Assay Buffer)**

IX. ASSAY PROCEDURE

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

1. Dilute the 10X Wash Buffer concentrate 10 fold by mixing the entire contents of each bottle of Wash Buffer with 450 mL deionized water (dilute both bottles with 900 mL deionized water).
2. Remove the required number of strips from the Microtiter Assay Plate. Assemble the strips in an empty plate holder and wash each well 3 times with 300 μ L of diluted Wash Buffer per wash. 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 in duplicate 50 μ L of 1:1 diluted Matrix Solution to blank wells, Standard wells, and Quality Control wells (**Do not use matrix if testing TISSUE CULTURE samples –substitute with 50 μ L Assay Buffer**).
4. Add in duplicate 50 μ L of Assay Buffer to blank wells and sample wells.
5. Add in duplicate 50 μ L FGF-23 Standards in the order of ascending concentration to the appropriate wells. Add in duplicate 50 μ L QC1 and 50 μ L QC2 to the appropriate wells. Add 50 μ L of the unknown 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 shake 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 μ L per well per wash. Decant and tap firmly after each wash to remove residual buffer.
9. Add 100 μ L Detection Antibody to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 1.0 hour on the microtiter plate shaker.
10. Remove sealer, decant solutions from the plate, and tap plate to remove the residual fluid.
11. Wash wells 3 times with diluted Wash Buffer, 300 μ L per well per wash. Decant and tap firmly after each wash to remove residual buffer.
12. Add 100 μ L 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.

IX. ASSAY PROCEDURE (continued)

13. Wash wells 3 times with diluted Wash Buffer, 300 μ L per well per wash. Decant and tap firmly after each wash to remove residual buffer.
14. Add 100 μ L 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 the FGF-23 standards with intensity proportional to increasing concentrations of FGF-23.

Note: Please be aware that the 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.

15. Remove sealer and add 100 μ L 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. Wipe the bottom of the microtiter plate to remove any residue prior to reading on plate reader. Read absorbance at 450 nm and 590 nm 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 the highest FGF-23 standard should be approximately 2.0 - 3.0, or not to exceed the capability of the plate reader used.

Assay Procedure for Rat FGF-23 ELISA Kit (Cat. # EZRFGF23-42K)

	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6-8	Step 9	Step 9-11	Step 12	12-13	Step 14	Step 14	Step 15	Step 15
Well #	Dilute each bottle of 10X Wash Buffer with 450 mL Deionized Water.	Wash plate 3X with 300 µL Wash Buffer. Remove residual buffer by tapping smartly on absorbent towels.	Matrix Solution	Assay Buffer	Standards/ Controls/ Samples	Seal, Agitate, Incubate 1.5 hours at Room Temperature. Wash 3X with 300 µL Wash Buffer	Detection Antibody	Seal, Agitate, Incubate 1.0 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			50 µL	50 µL	0 µL		100 µL		100 µL		100 µL			
C1, D1			50 µL	0 µL	50 µL of Tube 5									
E1, F1			50 µL	0 µL	50 µL of Tube 4									
G1, H1			50 µL	0 µL	50 µL of Tube 3									
A2, B2			50 µL	0 µL	50 µL of Tube 2									
C2, D2			50 µL	0 µL	50 µL of Tube 1									
E2, F2			50 µL	0 µL	50 µL of Reconstituted Standard									
G2, H2			50 µL	0 µL	50 µL of QC 1									
A3, B3			50 µL	0 µL	50 µL of QC 2									
C3, D3			0 µL	50 µL	50 µL of Sample									
E3, F3			0 µL	50 µL	50 µL of Sample									
G3, H3			0 µL	50 µL	50 µL of Sample									
A4, B4 ↓			0 µL	50 µL	50 µL of Sample									

X. MICROTITER PLATE ARRANGEMENT

Rat FGF-23 ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Tube 2	QC2									
B	Blank	Tube 2	QC2									
C	Tube 5	Tube 1	Sample 1									
D	Tube 5	Tube 1	Sample 1									
E	Tube 4	Reconstituted Standard	Sample 2									
F	Tube 4	Reconstituted Standard	Sample 2									
G	Tube 3	QC 1	Etc.									
H	Tube 3	QC 1	Etc.									

XI. CALCULATIONS

The dose-response curve of this assay fits best to a sigmoidal 4- or 5-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4- or 5-parameter logistic function

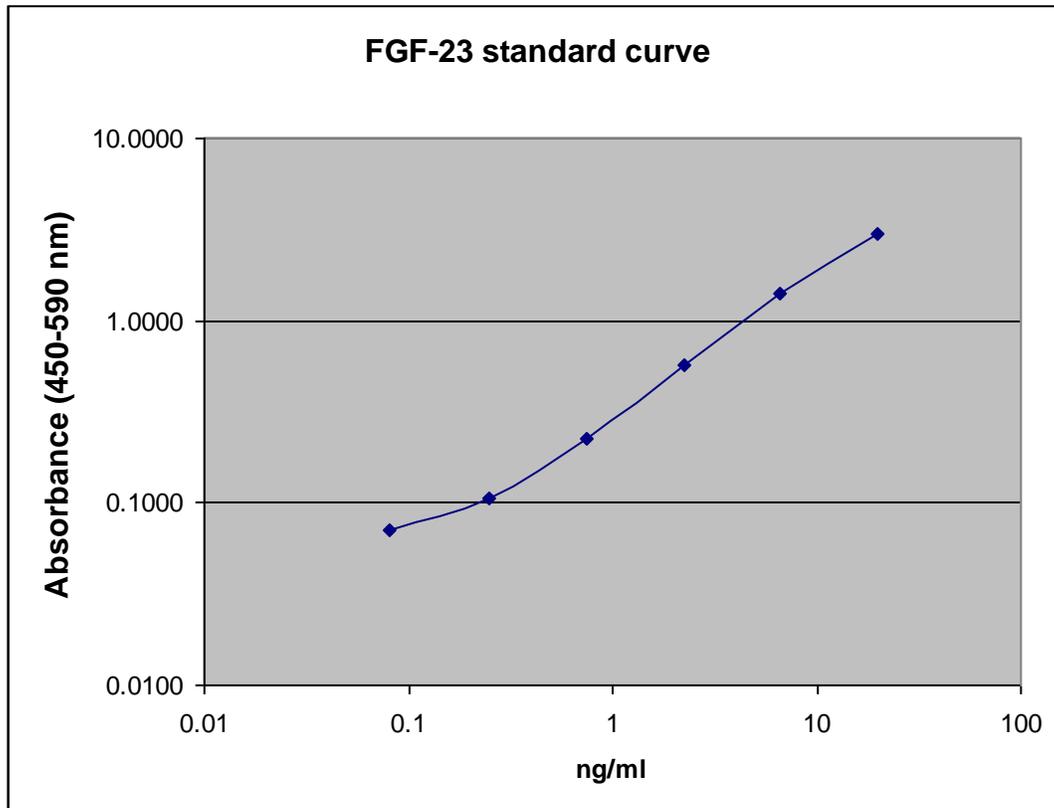
Note: When sample volumes assayed differ from 50 μL , an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 25 μL of sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 50 μL , compensate the volume deficit with Assay Buffer.

XII. INTERPRETATION

1. The assay will be considered accepted when all Quality Control values fall within the calculated Quality Control Range. If any QC's fall outside the control range, review results with a supervisor.
2. If the difference between duplicate results of a sample is $>15\%$ CV, repeat the sample.
3. The limit of sensitivity of this assay is 0.05 ng/mL Rat FGF-23 (50 μL sample size).
4. The appropriate range of this assay is 0.082 ng/mL to 20 ng/mL Rat FGF-23 (50 μL sample size). Any result greater than 20 ng/mL in a 50 μL sample should be diluted using Assay Buffer, and the assay repeated until the results fall within range.

XIII. STANDARD CURVE

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



XIV. ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of FGF-23 that can be detected by this assay is 0.05 ng/ml when using a 50 μ L sample size.

B. Specificity

The antibody pair used in this assay is specific to Rat FGF-23 and does not significantly cross-react to the following molecules/hormones tested: other FGFs

C. Precision

Intra-Assay Variation

Sample No.	Mean FGF-23 Levels (ng/mL)	Intra-Assay % CV
1	0.472	5.6
2	3.846	5.0

The assay variations of EMD Millipore Rat FGF-23 ELISA Kits were studied on two samples with varying concentrations of FGF-23. The mean intra-assay variation was calculated from the results of sixteen replicate determinations in each assay for the indicated samples.

Inter-Assay Variation

Sample No.	Mean FGF-23 Levels (ng/mL)	Inter-Assay % CV
1	0.480	8.1
2	3.556	5.5

The assay variations of EMD Millipore Rat FGF-23 ELISA Kits were studied on two samples with varying concentrations of FGF-23. The mean inter-assay variations of each sample were calculated from the results of six separate assays with duplicate samples in each assay.

XIV. ASSAY CHARACTERISTICS (continued)

D. Recovery

Spike & Recovery of Rat FGF-23 in Serum

Sample No.	FGF-23 Added ng/mL	Expected ng/mL	Observed ng/mL	% of Recovery
1	0		.436	
	0.25	0.686	.678	99
	0.74	1.176	1.089	93
	2.22	2.68	2.678	101
2	0		0.465	
	0.25	0.715	0.673	94
	0.74	1.205	1.175	98
	2.22	2.890	2.891	108
3	0		0.291	
	0.25	0.541	0.466	85
	0.74	1.200	0.852	83
	2.22	2.511	2.279	91
4	0		0.590	
	0.25	0.840	0.757	90
	0.74	1.330	1.129	85
	2.22	2.810	2.348	84
5	0		0.351	
	0.25	0.601	0.513	85
	0.74	1.091	0.862	79
	2.22	2.571	2.001	78

Varying amounts of Rat FGF-23 were added to five human serum or plasma samples and the FGF-23 content was determined in two separate assays. The % of recovery = observed FGF-23 concentrations/expected FGF-23 concentrations x 100%.

XIV. ASSAY CHARACTERISTICS (continued)

E. Linearity of Dilution

Sample No.	Volume Sampled	Expected ng/mL	Observed ng/mL	% Of Expected
1	50		1.546	
	25	0.773	0.576	75
	12.5	0.386	0.265	69
	6.25	0.193	0.152	79
2	50		1.551	
	25	0.724	0.724	93
	12.5	0.383	0.383	99
	6.25	0.247	0.247	127
3	50		1.609	
	25	0.711	0.711	88
	12.5	0.325	0.325	81
	6.25	0.181	0.181	90
4	50		1.977	
	25	0.747	0.747	76
	12.5	0.374	0.374	76
	6.25	0.223	0.223	90
5	50		1.163	
	25	0.653	0.653	112
	12.5	0.406	0.406	140

Five Rat samples with the indicated sample volumes were assayed. Required amounts of matrix solution were added to compensate for lost volumes below 50 μ L. The resulting dilution factors of 1.0, 2.0, 4.0, and 8.0 representing 50 μ L, 25 μ L, 12.5 μ L, and 6.25 μ L sample volumes assayed, respectively, were applied in the calculation of observed FGF-23 concentrations. % expected = observed/expected x 100%.

XV. QUALITY CONTROLS

The ranges for Quality Control 1 and 2 are provided on the card insert or can be located at the EMD MILLIPORE website www.emdmillipore.com using the catalog number as the keyword.

XVI. 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.

XVII. REPLACEMENT REAGENTS

Reagents	Cat. #
Rat FGF-23 ELISA Plate	EP42
10X HRP Wash Buffer Concentrate (50 mL)	EWB-HRP
FGF-23 Standards	E8042-K
FGF-23 Quality Controls 1 and 2	E6042-K
Matrix Solution	EMTX-PS6
Assay Buffer	EABPI
FGF-23 Detection Antibody	E1042
Enzyme Solution	EHRP-5
Substrate	ESS-TMB3
Stop Solution	ET-TMB

XVIII. ORDERING INFORMATION

To place an order:

To assure the clarity of your 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: (866) 441-8400
(636) 441-8400

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 ELISA products, please contact your technical 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
Norway	Norway@merckgroup.com
Spain	pedidos@merckgroup.com
Sweden	Kundservice@merckgroup.com
Switzerland	SZCustomerService@merckgroup.com
UK	UKCustomerService@merckgroup.com

XVIII. 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>