Mouse FGF-23

96-Well Plate

Cat. # EZMFGF23-43K

Mouse FGF-23 ELISA KIT

96-Well Plate

Cat. # EZMFGF23-43K

EMD Millipore Corporation • 3050 Spruce Street • St. Louis, Missouri 63103 USA
Toll Free U.S: 1-800 645-5476 • Fax: 1-800-645-5439
emdmillipore.com

I. INTENDED USE

This Mouse FGF-23 ELISA kit is used for the non-radioactive quantification of Mouse FGF-23 in serum, plasma, and tissue culture samples. This kit specifically measures native Mouse FGF-23. One kit is sufficient to measure 38 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 Mouse FGF-23 molecules from samples to the wells of a microtiter plate coated with a polyclonal goat anti- FGF-23 antibody, and binding of a second biotinylated polyclonal rabbit 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 Mouse 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 Mouse 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 Goat 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: 2.5 mL, bottle
Preparation: Dilute bottle with 2.5 mL deionized water 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 Rabbit 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

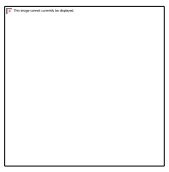
Sodium Azide or Proclin has been added to some reagents as a preservative. Although the concentrations are low, Sodium Azide and Proclin may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state,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

Full Hazardous Labels for components in this kit:

Ingredient, Cat #		Full Label	
Mouse Fibroblast Growth Factor-23 Quality Controls 1 & 2	E6043-K		**Warning.** Harmful if swallowed. Toxic to aquatic life with long lasting effects. Avoid release to the environment.
Mouse Fibroblast Growth Factor-23 Standard	E8043-K		**Warning.** Harmful if swallowed. Toxic to aquatic life with long lasting effects. Avoid release to the environment.
Matrix Solution	EMTX-RS2	No Symbol Required	Harmful to aquatic life with long lasting effects. Avoid release to the environment.
Stop Solution	ET-TMB		**Warning.** May be corrosive to metals.
10X HRP Wash Buffer Concentrate	EWB-HRP		**Warning.** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.

VI. MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes and Pipette Tips: 10 µL - 20 µL or 20 µL - 100 µL
2. Multi-Channel Pipettes and Pipette Tips: 5 µL ~ 50 µL and 50 µL ~ 300 µL
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^{o}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^{o}C for later use. For long-term storage, keep at -70 ^{o}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 six tubes 1, 2, 3, 4, 5, and 6. Add 0.200 mL Assay Buffer to each of the six tubes. Prepare serial dilutions by adding 0.100 mL of the reconstituted standard to Tube 1, mix well and transfer 0.100 mL of Tube 1 to Tube 2, mix well and transfer 0.100 mL of Tube 2 to Tube 3, mix well and transfer 0.100 mL of Tube 3 to Tube 4, mix well and transfer 0.100 mL of Tube 4 to Tube 5, mix well and transfer 0.100 mL of Tube 5 to Tube 6, 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°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	0.200 mL	0.100mL of Reconstituted Standard	X/3
Tube 2	0.200mL	0.100 mL of Tube 1	X/9
Tube 3	0.200 mL	0.100 mL of Tube 2	X/27
Tube 4	0.200 mL	0.100 mL of Tube 3	X/81
Tube 5	0.200 mL	0.100 mL of Tube 4	X/243
Tube 6	0.200 mL	0.100 mL of Tube 5	X/729

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 2.5 mL of deionized water and mix well. **(Do not use matrix if testing TISSUE CULTURE samples –substitute with 20 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 µL 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 20 µL of Matrix Solution to blank wells, Standard wells, and Quality Control wells **(Do not use matrix if testing TISSUE CULTURE samples –substitute with 20 µL Assay Buffer)**.

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

5. Add in duplicate 20 µL FGF-23 Standards in the order of ascending concentration to the appropriate wells. Add in duplicate 20 µL QC1 and 20 µL QC2 to the appropriate wells. Add 20 µL of the unknown samples in duplicate to the remaining wells.

6. Add 60 µL Assay Buffer to each well.

7. Cover the plate with plate sealer and incubate at room temperature for 1 hour on an orbital microtiter plate shaker set to shake at moderate speed, approximately 400 to 500 rpm.

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

9. 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.

10. Add 100 µL Detection Antibody to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 1 hour on the microtiter plate shaker.

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

12. 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.

IX. ASSAY PROCEDURE (continued)

13. Add 100 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.

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 100 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 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.

16. Remove sealer and add 100 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. Wipe the bottom of the microtiter plate to remove any residue prior to reading on plate reader. 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 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 Mouse FGF-23 ELISA Kit (Cat. # EZMFGF23-43K)

	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7-9	Step 10	Step 10-12	Step 13	Step 13-14	Step 15	Step 15	Step 16	Step 16
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	**Add 60 µL Assay Buffer to each well.**	**Seal, Agitate, Incubate 1.0 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			20 µL	20 µL	0 µL			100 µL		100 µL		100 µL		100 µL	
C1, D1			20 µL	0 µL	20 µL of Tube 6										
E1, F1			20 µL	0 µL	20 µL of Tube 5										
G1, H1			20 µL	0 µL	20 µL of Tube 4										
A2, B2			20 µL	0 µL	20 µL of Tube 3										
C2, D2			20 µL	0 µL	20 µL of Tube 2										
E2, F2			20 µL	0 µL	20 µL of Tube 1										
G2, H2			20 µL	0 µL	20 µL of Reconstituted Standard										
A3, B3			20 µL	0 µL	20 µL of QC 1										
C3, D3			20 µL	0 µL	20 µL of QC 2										
E3, F3			0 µL	20 µL	20 µL of Sample										
G3, H3			0 µL	20 µL	20 µL of Sample										
A4, B4 ↓			0 µL	20 µL	20 µL of Sample										

X. MICROTITER PLATE ARRANGEMENT

Mouse FGF-23 ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Tube 3	QC1	Etc.								
B	Blank	Tube 3	QC1	Etc.								
C	Tube 6	Tube 2	QC2									
D	Tube 6	Tube 2	QC2									
E	Tube 5	Tube 1	Sample 1									
F	Tube 5	Tube 1	Sample 1									
G	Tube 4	Reconstituted Standard	Sample 2									
H	Tube 4	Reconstituted Standard	Sample 2									

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

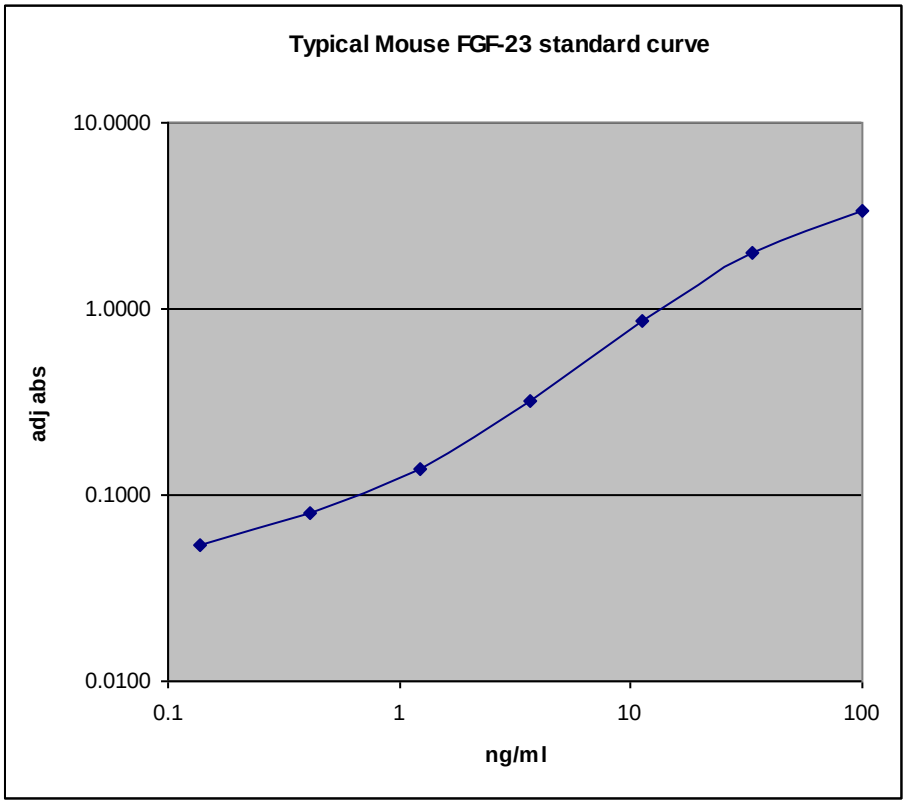
Note: When sample volumes assayed differ from 20 µL, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 10 µL of sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 20 µL, compensate the volume deficit 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 0.069 ng/mL Mouse FGF-23 (20 µL sample size).
4. The appropriate range of this assay is 0.137 ng/mL to 100 ng/mL Mouse FGF-23 (20 µL sample size). Any result greater than 100 ng/mL in a 20 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 0.069 ng/mL when using a 20 µL sample size.

B. Specificity

The antibody pair used in this assay is specific to Mouse 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	1.1	5.09
2	10.8	4.79

The assay variations of EMD Millipore Mouse 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	1.2	7.29
2	10.4	6.11

The assay variations of EMD Millipore Mouse 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 Mouse FGF-23 in Serum

Sample No.	FGF-23 Added ng/mL	Expected ng/mL	Observed ng/mL	% of Recovery
1	0		0.18	
	1.23	1.41	1.31	93
	3.7	3.88	3.66	94
	11.1	11.28	12.3	109
2	0		0.15	
	1.23	1.35	1.2	89
	3.7	3.85	3.61	94
	11.1	11.25	11.69	104
3	0		0.16	
	1.23	1.39	1.18	85
	3.7	3.86	3.72	96
	11.1	11.26	11.49	102
4	0		0.08	
	1.23	1.31	0.89	68
	3.7	3.78	2.71	72
	11.1	11.18	8.82	79
5	0		0.26	
	1.23	1.49	1.49	100
	3.7	3.96	3.81	96
	11.1	11.36	10.79	95

Varying amounts of Mouse FGF-23 were added to five human serum 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	20		6.74	
	10	3.37	2.94	87
	5	1.685	1.42	84
	2.5	0.842	0.67	80
2	20		5.3	
	10	2.65	2.87	108
	5	1.325	1.22	92
	2.5	0.663	0.62	94
3	20		5.81	
	10	2.905	2.91	100
	5	1.453	1.48	102
	2.5	0.726	0.71	98
4	20		6.7	
	10	3.35	3.23	96
	5	1.675	1.46	87
	2.5	0.837	0.69	82
5	20		6.78	
	10	3.39	3.13	92
	5	1.695	1.46	86
	2.5	0.847	0.64	76

Five Mouse serum or plasma samples with the indicated sample volumes were assayed. Required amounts of matrix solution were added to compensate for lost volumes below 20 µL. The resulting dilution factors of 1.0, 2.0, 4.0, and 8.0 representing 20 µL, 10 µL, 5 µL, and 2.5 µL 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. #
Mouse FGF-23 ELISA Plate	EP43
10X HRP Wash Buffer Concentrate (50 mL)	EWB-HRP
FGF-23 Standards	E8043-K
FGF-23 Quality Controls 1 and 2	E6043-K
Matrix Solution	EMTX-RS2
Assay Buffer	EABPI
FGF-23 Detection Antibody	E1043
Enzyme Solution	EHRP-5
Substrate	ESS-TMB3
Stop Solution	ET-TMB

XVIII. ORDERING INFORMATION

To place an order or to obtain additional information about our immunoassay products, please contact your Customer Service or Technical Support Specialist.

Contact information for each region can be found on our website:

emdmillipore.com/contact

Conditions of Sale

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

Safety Data Sheets (SDS)

Safety Data Sheets for EMD Millipore products may be ordered by fax or phone or through our website at emdmillipore.com/msds.