XF Base Medium Data Sheet
100 mL

Product: **XF Base Medium 100 mL**
Minimal DMEM
(0 mM Glucose)

Part Number: 103193-100

XF Base Medium is for use with Seahorse Bioscience XF instruments, XF Assay Kit cartridges and XF Cell Culture Microplates. XF Base Medium has been developed to allow customization of substrates in your assay medium.

XF assays require a non-buffered medium to accurately measure the extracellular acidification rate (ECAR), and proton production rate (PPR). The constituents are based on Dulbecco's Modified Eagle's Medium (DMEM). No sodium bicarbonate (buffering agent), glucose, glutamine/GlutaMAX™, or sodium pyruvate is present allowing the user to specifically customize the assay medium for their experimental design.

XF Base Medium 100 mL Specifications

Volume:	100 mL
Osmolality:	291 mOsm/Kg H_2O
Endotoxin:	< 0.0052 EU/mL
Storage:	4°C
Shelf life:	18 months when stored at 4°C

Component	Final Concentration
Mg^{2+} (as $MgSO_4$)	0.8 mM
Ca^{2+} (as $CaCl_2$)	1.8 mM
NaCl	143 mM
KCl	5.4 mM
NaH_2PO4	0.91 mM
Phenol Red	3 mg/L

Other Constituents:

L-Arginine*HCl, L-Cystine*2HCl, Glycine, L-Histidine*HCl*H_2O, L-Isoleucine, L-Leucine, L-Lysine*HCl, L-Methionine, L-Phenylalanine, L-Serine, L-Threonine, L-Tryptophan, L-Valine, L-Tyrosine*2Na*2H_2O, Folic Acid, Riboflavin, D-Ca-Panthothenate, Choline Chloride, i-Inositol, Nicotinamide, Pyridoxine*HCl, Thiamine*HCl.

Preparation for use with XF^e, XFp and XF Analyzers

XF Base Medium 100 mL contains no glucose, sodium pyruvate, or glutamine/GlutaMAX.

To prepare the XF Base Medium for an assay take the following steps:

1. Warm XF Base Medium to 37°C in water bath.
2. Add sodium pyruvate to desired final concentration based on the following table:

Final Pyruvate Concentration	Grams of Dry Pyruvate per 100 mL	mL of 100 mM Na Pyruvate per 100 mL
0.5 mM	0.0055 g	0.5 mL
1.0 mM	0.0110 g	1.0 mL
2.0 mM	0.0220 g	not recommended
5.0 mM	0.0550 g	not recommended
10.0 mM	1.1100 g	not recommended

3. Add glucose to the desired final concentration based on the following table:

Final Glucose Concentration	Grams of Glucose per 100 mL	mL of 45% Glucose solution per 100 mL
2.5 mM	0.045 g	0.10 mL
5.5 mM	0.099 g	0.22 mL
11 mM	0.180 g	0.44 mL
25 mM	0.450 g	1.00 mL

4. Add GlutaMAX or glutamine to the desired final concentration based on the following tables:

Final GlutaMAX Concentration	mL of 100X (200 mM) GlutaMAX solution per 100 m L
1 mM	0.5 mL
2 mM	1.0 mL
4 mM	2.0 mL

Final Glutamine Concentration	Grams of Glutamine per 100 mL	mL of 200 mM Glutamine solution per 100 mL
1 mM	0.0146 g	0.5 mL
2 mM	0.0292 g	1.0 mL
4 mM	0.0584 g	2.0 mL

5. Adjust pH of medium to 7.4 using NaOH or HCl.

6. Filter sterilize using a 0.22 micron filter if sterility was compromised.

7. Store at 4°C. Re-adjustment of pH may be necessary if stored for longer than 7 days.

Using the XF Base Medium 100 mLwith the XF Glycolysis Stress Test Kit:

To use XF Base Medium with the XF Glycolysis Stress Test Kit, supplement the XF Base Medium 100 mL with 2 mM (final concentration) L-glutamine (see table above). Note that no glucose or pyruvate is added to the XF Glycolysis Stress Test medium. Warm the medium to 37°C and adjust pH to 7.4. The supplemented medium should then be sterile filtered, and stored at 4°C.

Buffer Capacity Based on Glucose Concentration

The buffer capacity of the XF Base Medium 100 mL was determined for the following concentrations of glucose, glutamine and pyruvate. Medium was prepared as described in "Preparation for use with XF^{e}, XFp, and XF Analyzers."

mM Na Pyruvate	0	0	1
mM Glutamine	0	2	2
0.0 mM Glucose	-	0.0007210	-
5.5 mM Glucose	0.0007070	0.0006860	0.0007210
11 mM Glucose	0.0006860	0.0006470	0.0007140
25 mM Glucose	0.0007039	0.0007453	0.0009901

Note: It is recommended that serum (e.g. FBS) be omitted from the final medium formulation, as it will affect the buffer capacity of the medium. If FBS is required for cell viability or phenotypic maintenance, it is recommended to use low amounts (~1% or less), and determine the resulting buffer capacity empirically for accurate PPR calculations.