

XF Assay Medium Data Sheet

Product: **XF Assay Medium
Modified DMEM
(0mM Glucose)**

Part Number: **100965-000** (1 liter)

Ordered as: **101022-100** (2x1 liters)

XF Assay Medium is for use in conjunction with the Seahorse Bioscience XF Analyzer instruments, XF Assay Kit cartridges and XF tissue culture microplates. XF assays require a non-buffered medium to accurately measure extra cellular acidification rate and proton production rate (ECAR and PPR) of cells growing in culture. The constituents are based on the formulation of Dulbecco's Modified Eagle's Medium, including 2 mM final of L-glutamine (as L-alanyl-glutamine). Please note that NO sodium bicarbonate (buffering agent), glucose, or sodium pyruvate is present.

Medium Specifications

Volume: **1 L**
Osmolality: **291 mOsm/Kg H₂O**
Endotoxin: **< 0.0052 EU/mL**
Storage: **2-8°C**
Expiration: **18 months when properly stored**

Medium Formulation

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 ₂ PO ₄	0.91mM
L-Ala-Gln (Glutamax)	2 mM
Phenol Red	15 mg/L

Other Constituents:

L-Arginine*HCl, L-Cystine*2HCl, Glycine, L-Histidine*HCl*H₂O, L-Isoleucine, L-Leucine, L-Lysine*HCl, L-Methionine, L-Phenylalanine, L-Serine, L-Threonine, L-Tryptophan, L-Valine, L-Tyrosine*2Na*2H₂O, Folic Acid, Riboflavin, D-Ca-Panthenate, Choline Chloride, i-Inositol, Nicotinamide, Pyridoxine*HCl, Thiamine*HCl.

Preparation for use in XF Assay

XF Assay Medium contains NO sodium pyruvate or glucose. To prepare medium for an XF assay, do the following:

1. Warm medium to 37°C in water bath.

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2. Add sodium pyruvate to desired final concentration based on the following table:

Final Pyruvate Concentration	Grams of Dry Pyruvate per 1 L	mL of 100 mM Na Pyruvate per 1 L
0 mM	0.00 g	0.0 mL
0.5 mM	0.055 g	5 mL
1.0 mM	0.110 g	10 mL
2.0 mM	0.220 g	20 mL

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

Final Glucose Concentration	Grams of Glucose per 1 L	mL of 45% Glucose solution
0.0 mM	0.00 g	0.0 mL
2.5 mM	0.45g	1.0 mL
5.0 mM	0.90g	2.0 mL
11 mM	1.80g	4.4 mL
25 mM	4.50g	10 mL

4. Adjust pH of medium to 7.4 using NaOH or HCl.
5. Filter sterilize if sterility was compromised.
6. Aliquot desired amount to use in XF assay. The remainder should be stored at 4°C. Re-adjustment of pH may be necessary if stored for longer than 7 days.

Buffer Capacity Based on Glucose Concentration

The buffer capacity of the XF Assay Medium was determined for the following concentrations of glucose. Medium was prepared as described in "Preparation for use in XF Assay."

Glucose Concentration	Buffer Capacity
0 mM	0.0009430
5.5 mM	0.0009469
11 mM	0.0009198
25 mM	0.0009381

Note that Seahorse Bioscience suggests 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, Seahorse Bioscience suggests using low amounts (~1% or less) and determining the resulting buffer capacity empirically for accurate PPR calculations.

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Corporate Headquarters