

## Preparation of Assay Media for Use in XF Assays

This basic procedure details the preparation of assay media for use with (1) XF Cell Mito Stress Test Kit, and (2) XF Glycolysis Stress Test Kit

Seahorse recommends the use non-buffered assay medium for XF assays to ensure accurate, functional measurements of metabolic phenotypes in an ambient environment. The optimal assay media for the XF Cell Mito Stress Test and the XF Glycolysis Stress Test are different. Both can be prepared starting with the XF Base Medium and adding different substrates as determined by your cell model. Substrate requirements are cell type specific and may need to be determined empirically. For the XF Cell Mito Stress Test, assay medium with user-added glucose, sodium pyruvate and glutamine is recommended (see 1). For the XF Glycolysis Stress Test, assay medium with user-added glutamine is recommended (see 2).

### 1. XF Cell Mito Stress Test Assay Medium

For one tissue culture plate: if rinsing manually, 100 mL of assay medium is sufficient; if rinsing using the XF Prep Station 200 mL of assay medium is sufficient.

#### Materials

	Source	Cat #
XF Base Medium	Seahorse Bioscience	102353-100
Glucose	Sigma	G7528
Sodium Pyruvate (powder)	Sigma	P5280
Sodium Pyruvate (liquid, 100 mM)	Sigma	S8636
L-Glutamine (200 mM)	Life Technologies	25030-081
0.2 µM Sterile Filter		
pH Meter		
1 N NaOH		

#### Method

1. Warm 100 mL XF Base Medium to 37°C.
2. Add glucose for the desired final concentration using the following table.

Final glucose concentration	Grams of glucose per 100 mL
2.5 mM	0.045 g
5.5 mM	0.10 g
10 mM	0.18 g
25 mM	0.45 g

3. Add the desired amount of sodium pyruvate using powder or liquid using one of the following tables.

Addition of sodium pyruvate from powder:

Final pyruvate concentration	Grams of pyruvate per 100 mL
0.5 mM	0.0055 g
1.0 mM	0.011 g
2.0 mM	0.022 g
5.0 mM	0.055 g
10.0 mM	0.110 g

Addition of sodium pyruvate from 100 mM liquid:

Final pyruvate concentration	mL of pyruvate solution per 100 mL
0.5 mM	0.5 mL
1.0 mM	1 mL
2.0 mM	2 mL

4. Add the desired amount of L-glutamine using the following table.

Addition of L-glutamine from 200 mM liquid:

Final glutamine concentration	mL of glutamine solution per 100 mL
1 mM	0.5 mL
2 mM	1 mL
4 mM	2 mL

5. Adjust pH to 7.4 using 1 N NaOH. Note: medium will respond quickly to NaOH, use small volumes and add slowly to adjust pH.
6. Filter Sterilize with a 0.2  $\mu$ M filter.
7. Keep the XF Cell Mito Stress Test Assay Medium at 37°C until ready to use.

## 2. XF Glycolysis Stress Test Assay Medium

For one tissue culture plate: if rinsing manually, 100 mL of assay medium is sufficient; if rinsing using the XF Prep Station 200 mL of assay medium is sufficient.

### Materials

	Source	Cat #
XF Base Medium	Seahorse Bioscience	102353-100
L-Glutamine (200 mM)	Life Technologies	25030-081
0.2 $\mu$ M Sterile Filter		
pH Meter		
1N NaOH		

### Method

1. Warm 100 mL of XF Base Medium to 37°C.
2. Add the desired amount of L-glutamine according to table below.

Final glutamine concentration	mL of L-glutamine solution per 100 mL
1 mM	0.5 mL
2 mM	1 mL
4 mM	2 mL

6. Adjust the pH to  $7.35 \pm 0.05$  using 1 N NaOH. Note: medium will respond quickly to NaOH, use small volumes and add slowly to adjust pH.
8. Filter sterilize with a 0.2  $\mu$ M filter.
9. Keep the XF Glycolysis Stress Test Assay Medium at 37°C until ready to use.