Preparation of XF assay media

Basic Procedure

This basic procedure details the preparation of assay media for use with Agilent Seahorse XF®96/ XF96, XF®24/ XF24, and XFp Analyzers and the following assay kits:

1. Cell Energy Phenotype Test
2. Cell Mito Stress Test
3. Mito Fuel Flex Test
4. Glycolytic Rate Assay
5. Glycolysis Stress Test

The use of non- or low-buffered assay medium is recommended for XF assays to ensure accurate, consistent functional measurements of metabolic activity. The assay medium compositions are slightly different depending on the assay kit used. All compositions can be prepared using one of the Agilent Seahorse XF Base Mediums and adding different substrates/buffer as determined by the specific assay design.

Please see the Agilent Seahorse XF Media and Buffer Selection Guide to determine the proper base media and buffer required for the assay to be performed: http://www.agilent.com/cs/library/selectionguide/public/5991-7878EN.pdf.

Equipment Required

1. Water bath at 37°C
2. 0.2 µm Sterile Filter
3. Calibrated pH meter
4. 1 N NaOH
Reagents Required (depending on the assay to be run)

### XF Assay Media Preparation for: Cell Energy Phenotype Test, Cell Mito Stress Test, Mito Fuel Flex Test

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Final Concentration</th>
<th>XF^a96/XF^a96 or XF^a24/XF^a24 (100 mL)</th>
<th>XFp (10 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agilent Seahorse XF Base Medium (DMEM)*</td>
<td>-</td>
<td>97.6 mL</td>
<td>9.76 mL</td>
</tr>
<tr>
<td>Glucose (2.5M)</td>
<td>10 mM</td>
<td>400 µL</td>
<td>40 µL</td>
</tr>
<tr>
<td>Sodium Pyruvate (100 mM solution)</td>
<td>1 mM</td>
<td>1 mL</td>
<td>100 µL</td>
</tr>
<tr>
<td>L-Glutamine (200 mM solution)</td>
<td>2 mM</td>
<td>1 mL</td>
<td>100 µL</td>
</tr>
</tbody>
</table>

* XF Base Medium DMEM without Phenol Red or RPMI without Phenol Red may be substituted for XF Base Medium (DMEM)

### XF Assay Media Preparation for: Glycolytic Rate Assay

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Final Concentration</th>
<th>XF^a96/XF^a96 or XF^a24/XF^a24 (100 mL)</th>
<th>XFp (10 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agilent Seahorse XF Base Medium DMEM or RPMI, without Phenol Red</td>
<td>-</td>
<td>97.1 mL</td>
<td>9.76 mL</td>
</tr>
<tr>
<td>Glucose (2.5M)</td>
<td>10 mM</td>
<td>400 µL</td>
<td>40 µL</td>
</tr>
<tr>
<td>Sodium Pyruvate (100 mM solution)</td>
<td>1 mM</td>
<td>1 mL</td>
<td>100 µL</td>
</tr>
<tr>
<td>L-Glutamine (200 mM solution)</td>
<td>2 mM</td>
<td>1 mL</td>
<td>100 µL</td>
</tr>
<tr>
<td>Agilent Seahorse 1 M Hepes</td>
<td>5 mM</td>
<td>500 µL</td>
<td>50 µL</td>
</tr>
</tbody>
</table>

### XF Assay Media Preparation for: Glycolytic Stress Test

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Final Concentration</th>
<th>XF^a96/XF^a96 or XF^a24/XF^a24 (100 mL)</th>
<th>XFp (10 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agilent Seahorse XF Base Medium (DMEM)*</td>
<td>-</td>
<td>99.0 mL</td>
<td>9.9 mL</td>
</tr>
<tr>
<td>L-Glutamine (200 mM solution)</td>
<td>2 mM</td>
<td>1 mL</td>
<td>100 µL</td>
</tr>
</tbody>
</table>

* XF Base Medium DMEM without Phenol Red or RPMI without Phenol Red may be substituted for XF Base Medium (DMEM)

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Method

1. Warm appropriate type and volume of Agilent Seahorse XF Base Medium to 37°C. 100 mL is sufficient for one XF-96/XF96 or one XF-24/XF24 plate. 10 mL is sufficient for one XFp plate.
2. Add the indicated volumes of reagents indicated in the table(s) above, based on assay type and XF platform used.
3. Adjust pH value of the media to 7.4 using 1 N NaOH. Note: pH value will change quickly upon addition of NaOH, use small volumes and add slowly to adjust pH value.
4. Sterilize assay media with a 0.2 µm filter.
5. Incubate the Assay Medium at 37°C until ready for use.

Notes and Suggestions

1. Glucose, glutamine, and pyruvate are the most commonly added substrates to cell culture and XF assay medium. Oxidation and/or utilization of these substrates is required for XF assays. In XF Assays, these substrates are typically provided in saturating concentrations (reflected in the tables above) to ensure that they are not limiting the rates of respiration (OCR) and/or extracellular acidification (ECAR).
2. It is recommended that substrate concentrations of 10 mM glucose, 1 mM pyruvate and 2 mM glutamine are used in initial XF assays (except the Glycolytic Stress Test, 2 mM glutamine only). Note, however, that substrate requirements can be cell-type specific and may need to be determined empirically.
3. It is recommended that glutamine-alanine dipeptide (e.g. GlutaMAX™) NOT be used as a glutamine source in XF assays.
4. It is recommended to incubate assay medium at 37°C for not more than 4 hours, as substrates, such as glutamine, can degrade.

Learn more

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