

Agilent Seahorse XF Media Selection Guide



Agilent Seahorse XF Media are specially formulated for use in XF Assays and are recommended for best results. XF Media are based on standard cell culture media compositions (DMEM or RPMI), but vary from typical growth/culture media formulations in a few key ways:

- **No bicarbonate and low buffering capacity:** to improve the detection of extracellular acidification.
- **No supplements:** allowing specific customization of the assay medium.
- **No phenol red:** to allow for the most accurate and precise measurement of absolute pH values.

Agilent Seahorse offers buffered XF media with a pre-adjusted pH of 7.4 at 37 °C (part numbers 103575-100 and 103576-100). These media contain a low amount of HEPES buffer. When used with compatible XF supplements (e.g., XF glucose solution, XF pyruvate solution, or XF glutamine solution) at recommended concentrations, there is no need to adjust the pH of the media; this simplifies the workflow and reduces the time needed for assay preparation. These pH-ready media also have consistent buffering capacities, leading to more consistent assay data across experiments.

Best practices for preparing Agilent Seahorse XF Assay Media using XF DMEM or RPMI media, pH 7.4

1. Transfer a sufficient volume of XF DMEM or RPMI media, pH 7.4 to a new sterile bottle on the day of use.
Note: It is recommended to not warm up the entire bottle of medium if a smaller volume is needed, and to tighten the bottle cap after each use to maintain the pH value.
2. Add desirable amounts of XF supplements/substrates.
Note: XF supplements must be used at the recommended concentration range to ensure a proper final pH in assay media. Proper medium pH is not guaranteed if brand supplements from other suppliers are used. Recommended supplement concentrations are 0 to 10 mM for glucose, 0 to 1 mM for pyruvate, and 0 to 2 mM for glutamine. Specific supplement concentrations are assay-dependent. See [Procedures for Preparing XF Assay Media](#) and [XF Assay Kit User Guides](#) for more information. Filter sterilization of the final assay medium is not required if the sterility of the medium and all supplements has not been compromised.
3. Warm the medium to 37 °C. The assay medium is ready to use (no pH-adjustment is necessary).

Best practices for preparing Agilent Seahorse XF Assay Media using media that require pH adjustment

1. Transfer a sufficient volume of XF medium to a new bottle on the day of use.
2. Add desirable amounts of supplements/substrates.
Note: 5 mM HEPES buffer must be added to the Base Medium for the Glycolytic Rate Assay, Real-Time ATP Rate Assay, and T Cell Metabolic Profiling Assays.
3. Warm the medium to 37 °C.
4. Adjust the pH to 7.4 ± 0.1.
5. Filter-sterilize the medium after adjusting the pH value. The assay medium is ready to use.

Agilent Seahorse XF Media, Buffer, and supplement products

Part No.	Product Name	Size	Core Formula	Phenol Red	Note
103575-100	Seahorse XF DMEM Medium, pH 7.4	500 mL	DMEM	No	Suitable for all XF assays. Contains HEPES.
103576-100	Seahorse XF RPMI Medium, pH 7.4	500 mL	RPMI	No	Suitable for all XF assays. Contains HEPES.
103335-100	Seahorse XF Base Medium (without Phenol Red)	500 mL	DMEM	No	Suitable for all XF assays. 5 mM HEPES is required for some assays.
103577-100	Seahorse XF 1.0 M Glucose Solution, 50 mL	50 mL	N/A	No	Compatible with all XF media.
103578-100	Seahorse XF 100 mM Pyruvate Solution, 50 mL	50 mL	N/A	No	Compatible with all XF media.
103579-100	Seahorse XF 200 mM Glutamine Solution, 50 mL	50 mL	N/A	No	Compatible with all XF media.

All Seahorse XF Media/Buffer/Supplements are endotoxin tested and should be stored at 4 °C, except for glutamine solution which should be stored at –20 °C

Agilent Seahorse XF Media, Buffer, and supplement products

Part No.	Product Name	Real-Time ATP Rate Assay	Cell Mito Stress Test	Glycolytic Rate Assay	Glycolysis Stress Test	Mito Fuel Flex Test	Substrate Oxidation Stress Test	Palmitate Oxidation Stress Test	T Cell Metabolic Profiling Assays
103575-100	Seahorse XF DMEM Medium, pH 7.4	●	●	●	●	●	●	●	●
103576-100	Seahorse XF RPMI Medium, pH 7.4	✓	✓	✓	✓	✓	✓	✓	✓
103335-100	Seahorse XF Base Medium (without Phenol Red)	✓●	✓	✓●	✓	✓	✓	✓	✓●

● Recommended

✓ Compatible

● Requires addition of HEPES

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This information is subject to change without notice.

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