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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 composition (DMEM or RPMI), but varies from typical growth/culture medium 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.
- **Low or no phenol red:** to allow for the most accurate and precise measurement of absolute pH values.

Agilent Seahorse now offers buffered XF media with pre-adjusted pH of 7.4 at 37 ºC (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 and XF glutamine solution) at recommended concentrations, there is no need to adjust pH of the media, thus, simplifying workflow and reducing time needed for assay preparation. These pH-ready media also have consistent buffering capacity, 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 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 media if a smaller volume is needed, and to tighten the bottle cap after each use to maintain pH value.
2. Add desirable amounts of XF supplements/substrates.
   Note: XF supplements must be used at recommended concentration range to ensure a proper final pH in assay media. Proper media pH is not guaranteed if other brand supplements from other suppliers are used. Recommended supplement concentrations are 0-10 mM for glucose, 0-1 mM for pyruvate and 0-2 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 media is not required if sterility of media and all supplements has not been compromised. Please do not add additional HEPES to these media.
3. Warm medium to 37 ºC. 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.
   For assay media used in XF Glycolytic Rate Assay and XF Real-Time ATP Rate Assay, small amount of HEPES buffer is required. If using media that contains no HEPES, 5 mM HEPES should be added to DMEM-based medium and 1 mM HEPES should be added to RPMI-based medium, prior to pH adjustment. Check product label or product data sheets to find out if a medium to be used contains HEPES and proceed accordingly.
3. Warm medium to 37 ºC.
4. Adjust pH to 7.4 +/- 0.1.
5. Filter-sterilize the medium after adjusting 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	Recommended for all XF assays. Contains 5mM HEPES.
103576-100	Seahorse XF RPMI Medium, pH 7.4	500 mL	RPMI	No	Recommended for all XF assays. Contains 1mM HEPES.
103335-100	Seahorse XF Base Medium (without Phenol Red)	500 mL	DMEM	No	Suitable for all XF assays. 5 mM HEPES is required to be added for Glycolytic Rate Assay.
103336-100	Seahorse XF RPMI Medium (without Phenol Red)	500 mL	RPMI	No	Suitable for all XF assays. 1 mM HEPES is required to be added for Glycolytic Rate Assay.
102353-100	Seahorse XF Base Medium	2 x 1L	DMEM	Yes	Suitable for all XF assays except for Glycolytic Rate Assay and XF Real-time ATP Rate Assay.
103334-100	Seahorse XF Base Medium, 500 mL	500 mL	DMEM	Yes	Suitable for all XF assays except for Glycolytic Rate Assay and XF Real-time ATP Rate Assay..
103193-100	Seahorse XF Base Medium, 100 mL	100 mL	DMEM	Yes	Suitable for all XF assays except for Glycolytic Rate Assay and XF Real-time ATP Rate Assay.
103337-100	Seahorse 1 M HEPES, 30 mL	30 mL	n/a	No	Required for Glycolytic Rate Assay if using 103335-100 or 103336-100.
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.

Note: All Seahorse XF Media/Buffer/Supplements are endotoxin tested and should be stored at 4 ºC, except for glutamine solution which should be stored at -20 ºC

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Part No	Product Name	Real-Time ATP Rate Assay	Cell Mito Stress Test	Glycolytic Rate Assay	Glycolysis Stress Test	Cell Energy Phenotype Test	Mito Fuel Flex Test
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)	✓●	✓	✓●	✓	✓	✓
103336-100	Seahorse XF RPMI Medium (without Phenol Red)	✓●	✓	✓●	✓	✓	✓
102353-100 103334-100 103193-100	Seahorse XF Base Medium, 2 x 1 L Seahorse XF Base Medium, 500 mL Seahorse XF Base Medium, 100 mL	●	✓	●	✓	✓	✓

● Recommended. No addition of HEPES is needed.
✓ Compatible
● Not Compatible
● Require addition of HEPES

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