Media Protocol

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Products:

Agilent Seahorse XF DMEM Medium, pH 7.4
Agilent Seahorse XF RPMI Medium, pH 7.4

Part Number: 103575-100 and 103576-100

Agilent Seahorse XF DMEM and RPMI Media, pH 7.4 (part numbers 103575-100 and 103576-100) are pre-adjusted pH media and are central components of a complete system for XF assay medium preparation. When used in conjunction with compatible XF supplements (e.g. XF glucose solution, XF pyruvate solution and/or XF glutamine solution) at recommended concentration ranges, there is no need to adjust pH of the final assay media, thus simplifying workflow and reducing time needed for XF assay preparation. These pre-adjusted pH media contain a low amount of HEPES (5 mM for DMEM-based medium and 1 mM for RPMI-based medium) and provide consistent buffering capacity, leading to more uniform assay data across experiments.

The procedure below describes the steps to use these media in preparation for XF assay media.

Equipment and Reagents required:

- 37 ºC water bath
- XF 1.0 M Glucose Solution (p/n 103577-100)
- XF 100 mM Pyruvate Solution (p/n 103578-100)
- XF 200 mM Glutamine Solution (p/n 103579-100)

Procedure:

1. Transfer a sufficient volume of XF DMEM or RPMI media, pH 7.4 to a sterile bottle on the day of use.
2. Add proper volumes of compatible XF supplements to achieve the desired final concentrations. Recommended concentration ranges are 0-10 mM for glucose, 0-1 mM for pyruvate and 0- 2 mM for glutamine.
3. Warm medium to 37 ºC in water bath. The assay medium is ready to use (no pH-adjustment is necessary).

Tips and Best Practice:

1. On average, 100 mL assay medium is needed for running one XF assay (96- or 24-well format) and 10 mL for one XFp assay.
2. These media already contains low amount of HEPES to provide some buffering capacity, leading to more consistent results. Please do not add additional HEPES to these media for XF assays.
3. The XF DMEM and RPMI media products contain 500 mL per bottle. It is recommended to warm up only the volume required for the assay, but not the entire bottle of media. Bottle cap must be tightened securely after each use to maintain pH value.
4. The shelf life for these media in a sealed bottle is 12 months from the date of manufacture. It is recommended that entire bottle content be used within one month after initial use. pH value is not guaranteed for media opened for longer than 1 month.
5. Compatible XF supplements must be used at the recommended concentration range to ensure a proper final pH in assay media. Proper media pH is not guaranteed if supplements from other suppliers are used. Recommended supplement concentration ranges 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.
6. Filter sterilization of the final assay media is not required if sterility of media and all supplements has not been compromised.
7. XF DMEM Medium, pH 7.4 and XF RPMI Medium, pH 7.4 will be added to Media Catalogue in Wave Software 2.6. If using Wave 2.5 or previous versions, select Glycolytic Rate Assay Medium (DMEM- Based) or Glycolytic Rate Assay Medium (RPMI-based) as **Assay Medium Type** in **Assay Media Definition** in order to assign Buffer Factor and display PER data.

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