Training Guide

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Knowing your Cells of Interest: Advice and Suggestions for a Successful XF Experience

Growth/Proliferation rate of cells

- Understanding the proliferation rate of the cells provides several advantages; initially, this information helps in estimating the number of cells seeded per well in the Seahorse XF/XFp Cell Culture Microplates/Miniplates, and subsequently allows for approximation of final cell number after a period of growth.
- It is especially important to know the proliferation rate when interventions (e.g. genetic modifications, chronic drug treatments) are being introduced, as these often result in changes in cell growth rates and thus must be taken into consideration (i.e. normalization) when analyzing and interpreting XF data in your research.

Proper Cell Culture and Sub-culture

It is suggested to adhere to best tissue culture practices when preparing cells for XF assays. While different cell types can have markedly diverse growth conditions, several generic parameters do apply:

- Tracking passage number of cells.
- Ensuring that cells are maintained at or below a proper density (if required) during subculture.
- Providing proper maintenance or differentiation media at regular intervals.

Excellent sources of information on growth and maintenance of cells include ATCC and other cell repositories and distributors.

Best practices for cell seeding

- All Seahorse XF/XFp Cell Culture Microplates/Miniplates are plasma treated, and thus can be used like any tissue culture plate. It is recommended to seed adherent cell lines one day prior to the XF assay (though this can for certain cell types or applications).
- Primary cells may be seeded directly into Seahorse XF/XFp Cell Culture Microplates/Miniplates and allowed to grow for the requisite number of days before the assay. Cell maintenance (incubator conditions, changing media) should be performed as usual for that cell type.
- All formats of Seahorse XF/XFp Cell Culture Microplates/Miniplates may be coated with adherents (e.g. Cell Tak™, poly-D-Lysine, collagen, etc.) if necessary to facilitate cell attachment. Methods for coating plates may be found in Module 3: Performing the XF Assay.
- Follow the instructions in Agilent Seahorse XF Training Module 2: The Day Prior to the XF Assay, for optimal cell seeding techniques to ensure consistent cell density and proliferation rates from well-to-well and plate-to-plate.

Considerations for XF Assay Media Composition

- Agilent offers a variety of media formulated specifically for XF assays: Seahorse XF Media is either DMEM or RPMI based, with or without phenol red, and without sodium bicarbonate, glucose, pyruvate or glutamine.
- The initial omission of substrates from the media allows the user to customize the media composition based on the assay performed. Please consult the XF Assay Media and Buffer Selection Guide for choosing the proper assay media for your XF experiment, and consult the specific XF assay kit manual/application note for proper XF assay media preparation, respectively.
- Most XF assays use standardized concentrations of added glucose (10 mM), pyruvate (1 mM) and glutamine (2 mM), which are saturating in most cell types. This should be considered a starting point, and some alterations may be needed, depending on the goal of the investigation.
- Note that some cell types have specific XF assay media requirements (e.g. primary cortical neurons use artificial cerebral spinal fluid, no glutamine).
- The Agilent Seahorse Cell Reference Database, Publication Database, and Seahorse XF Assay Guides & Templates provide cell/assay specific information regarding XF assay media composition. as well as initial cell density. Please note that cell density information should be taken as a starting point, and it is encouraged that the user performs an initial cell density optimization for each cell type tested.

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