Analyzing cells with biocrates kits

Content

1 Introduction

An extraction protocol is provided below for analyzing cell samples with the following kits:

- MxP® Quant 500 XL
- MxP® Quant 500
- AbsoluteIDQ® p180
- AbsoluteIDQ® p400 HR
- MxP® Quant HR Xpress™
- AbsoluteIDQ® Bile Acids

Please note that only feasibility tests have been carried out, and the kits have not been validated with cell samples. The protocol below is a recommendation based on our experience and can be modified according to your needs or ideas. We always recommend performing pilot tests with representative cell samples before starting a larger study. The results may depend on the nature, quality and preparation of the samples.

2 References

For additional protocols and impressions, please refer to Andresen et al.:
https://www.biorxiv.org/content/10.1101/2021.12.15.470649v1

In this publication, ten different extraction methods of different complexity were applied and compared to both adherent (HEK) and non-adherent (HL60) cells. Among the protocols that are least time-consuming and most suitable for routine use, isopropanol has proven to be the most efficient solvent, yielding the highest number and broadest range of metabolites. If only polar compounds are of interest, ethanol/phosphate buffer 0.01 M (85:15 v/v) is recommended.

3 Preparing extraction solvents

Extraction solvent	Description
Isopropanol if polar and unpolar metabolites are of interest	Pure isopropanol, LC-MS grade
Ethanol/phosphate buffer if only polar metabolites are of interest or if the AbsoluteIDQ® Bile Acids kit is used	Ethanol/phosphate buffer* (85:15 v/v), combine – 85 mL of HPLC grade ethanol with – 15 mL of phosphate buffer, 0.01 M*

* Recommended: Sigma, P5244 (0.1 M, pH = 7.5 at 25 °C); 1:10 diluted

4 WebIDQ software and workflow differences

The table below describes the steps that are different to the regular workflow. All steps not mentioned here are unchanged and performed according to the user manual of the kit used.

Step	Instruction
1	Select the material "Cells" (or similar that applies) when registering cell samples in the LIMS module of WebIDQ.
2	Use the extraction solvent as zero sample. In the **Zeros** tab of the **Worklist generation** window, link the extraction solvent used.

5 Sample collection

Please refer to biocrates document **Technical guide-Cell sampling (v#).pdf** (available from your biocrates representative).

6 Sample preparation

Step	Instruction
1	Thaw frozen cell pellets on ice (approx. 3 x 10^6 cells).
2	Equilibrate the extraction solvent to freezer temperature (approx. -18 °C).
3	MxP® Quant 500 XL kit: Add **40 µL** of ice-cold extraction solvent **per 10^6 cells** to resuspend cell pellets. Any other kit: Add **25 µL** of ice-cold extraction solvent **per 10^6 cells** to resuspend cell pellets.
4	Sonicate resuspended cells in ice bath for 3 min.
5	Snap-freeze sonicated samples in liquid nitrogen for 30 sec.
6	Repeat sonication/freezing/sonication 2 times (in total 3 freeze-thaw cycles).
7	Centrifuge samples at 2 °C for 5 min at 31,500 x g.
8	Transfer the supernatant to a new and labeled vial.
9	Keep the extract on ice for immediate kit preparation or store at –80 °C.
10	For kit preparation, add 10 µL of the extract to the kit plate and follow the regular kit user manual.

Need any help? Please contact us: support@biocrates.com