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Case study: customized media for protein expression studies

Custom expression medium for Gibco™ Expi293™ Cells enabled a study of the conformational space at specific sites of the cannabinoid receptor 2

In 2023, Becker-Baldus et al. used Gibco™ Expi293™ Expression Medium and dynamic nuclear polarization (DNP)-enhanced magic angle spinning (MAS) nuclear magnetic resonance (NMR) spectroscopy to study the conformational space at specific sites of the cannabinoid receptor 2 (CB2) [1]. Media and transfection reagents from Thermo Fisher Scientific allowed the optimization of sample preparation, uncovering differences in the availability of the CB2 protein backbone at different receptor sites. Specifically, the authors used a custom amino acid–depleted Expi293 expression and complexation medium manufactured by Thermo Fisher to express methionine/valine– and methionine/arginine–labeled CB2. Gibco™ Expi293F™ GnTI– Cells (Cat. No. A39240) were initially grown in complete Expi293 Expression Medium (Cat. No. A14351), then resuspended in the amino acid–depleted medium on the day of transfection. Cells were transfected using the Gibco™ ExpiFectamine™ 293 Transfection Kit (Cat. No. A14525) with the CB2-expression plasmid and harvested 48 hr post-transfection by centrifugation. The yield of labeled CB2 was the same or better compared to reported expression levels of isotope-labeled G-protein–coupled receptors (GPCRs) in *Escherichia coli* and *Pichia pastoris*. Results support conformational variation between different sites at CB2, which depends on the ligand type (Figure 1), advancing a crucial understanding of the activation and signaling of GPCRs.

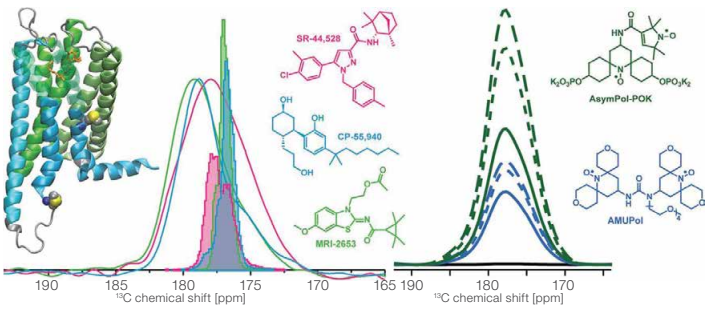

Figure 1. Conformational space at different sites of CB2. Becker-Baldus J et al. used Expi293 Expression Medium and transfection reagents from Thermo Fisher, and DNP-enhanced MAS NMR spectroscopy with AsymPol-POK as the polarizing agent, to study the conformational space at specific CB2 sites [1]. The reagents offered by Thermo Fisher allowed the optimization of sample preparation, uncovering that the conformational space available to the CB2 backbone is different at different receptor sites and depends on the binding ligand. Image from Becker-Baldus J et al. [1], used without changes, under the terms of the Creative Commons Attribution 4.0 International License (CC BY 4.0) (creativecommons.org/licenses/by/4.0/).

Case study: classical basal customized media for metabolic studies

Custom DMEM/F-12 medium enabled a study of quantitative determinants of aerobic glycolysis that identifed flux through the enzyme GAPDH as a limiting step

In this study, Shestov et al. sought to develop a computational method for analyzing the quantitative changes to metabolism necessary for aerobic glycolysis, or the Warburg Effect, a hallmark of cancer cells [2]. One part of the study involved exploring the metabolic responses of human mammary epithelial MCF-10A cells under varying nutrient conditions, specifically focusing on the NADH/NAD⁺ ratio, in order to confirm a computational model of glycolysis the researchers developed. The researchers generated MCF-10A cells that stably expressed the Peredox-NLS biosensor to visualize and quantify intracellular NADH/NAD⁺ levels using fluorescence imaging techniques. Key findings revealed that the NADH/NAD⁺ ratio distribution changed in response to differences in glucose availability, experimentally reflecting varied responses that epithelial cells may demonstrate in response to differences in nutrient availability. These observations provided experimental confirmation to the scientists that the glycolysis model they generated was reliable to provide even more insight into important metabolic functions.

Custom Gibco™ DMEM/F-12 medium was instrumental in enabling these study results by providing a precisely controlled environment devoid of glucose, allowing for the supplementation of specific nutrient levels as needed. This tailored medium facilitated the accurate assessment of how varying glucose concentrations influenced the NADH/NAD⁺ ratio distributions in MCF-10A cells (Figure 2). This level of control ensured that the observed metabolic responses were directly attributable to the manipulated nutrient conditions, thereby validating the experimental outcomes and enhancing the reliability of the findings.

References

1. Becker-Baldus J, Yeliseev A, Joseph TT, et al. (2023) Probing the conformational space of the cannabinoid receptor 2 and a systematic investigation of DNP-enhanced MAS NMR spectroscopy of proteins in detergent micelles. *ACS Omega* 8(36):32963-32976. doi: 10.1021/acsomega.3c04681. ncbi.nlm.nih.gov/pmc/articles/PMC10500644/
2. Shestov AA, Liu X, Ser Z, et al. (2014) Quantitative determinants of aerobic glycolysis identify flux through the enzyme GAPDH as a limiting step. *Elife* 3:e03342. doi: 10.7554/eLife.03342. pubmed.ncbi.nlm.nih.gov/25009227/

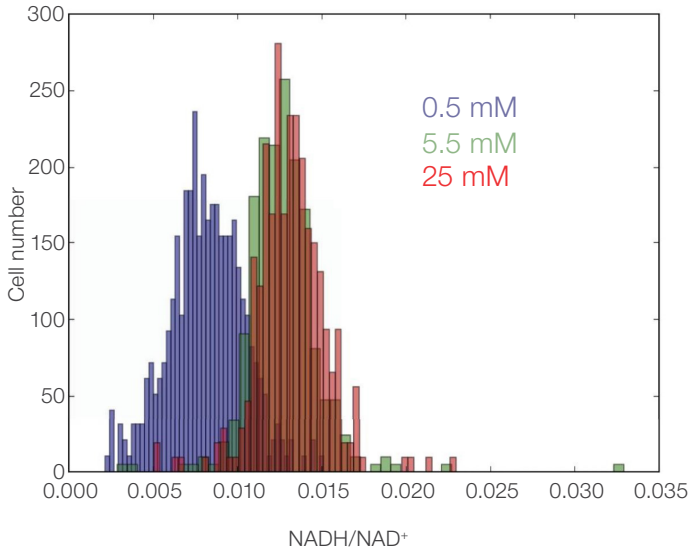

Figure 2. A quantitative model and statistical simulation method capture the diversity of metabolic states observed in tumor and proliferating cells. Measured values of the NADH/NAD⁺ ratio across a population of MCF-10A breast epithelial cells are shown. Three values of glucose concentration were considered (0.5 mM, 5.5 mM, and 25 mM). Figure from Shestov AA et al. [2], used without changes, under the terms of the Creative Commons Attribution 4.0 International License (CC BY 4.0) (creativecommons.org/licenses/by/4.0/).

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