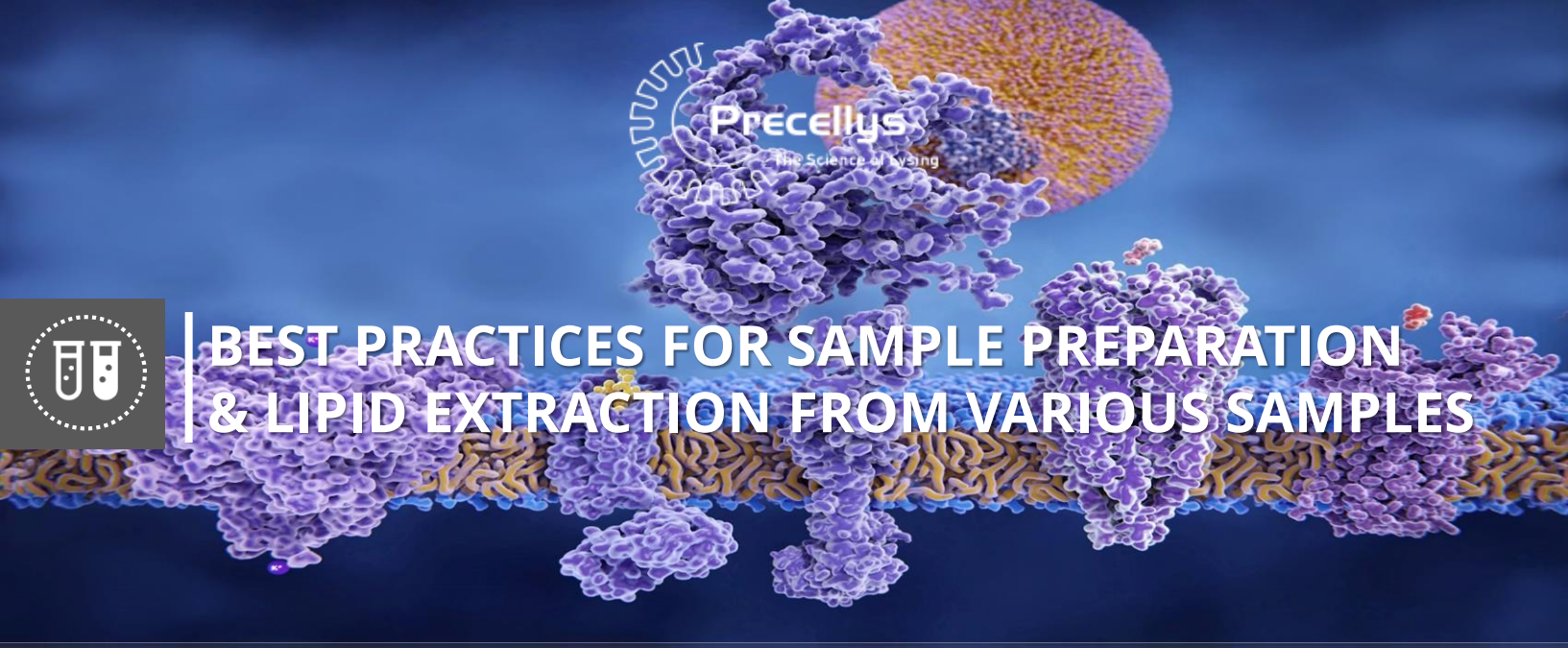


BEST PRACTICES FOR SAMPLE PREPARATION & LIPID EXTRACTION FROM VARIOUS SAMPLES

Lipids play a structural role in organisms, forming cells and organelles' membranes. Not only do they store energy, but recent studies also showed their role as first and second messengers in signal transduction. As they are implicated in widespread pathologies such as cancer or inflammation, they have become a major concern for the scientific community.

Lipid extraction for analysis can be challenging due to their structure and composition, in various biological materials. Different kinds of lipids, such as complex organic compounds, fats, oils, waxes and even hormones exist in living cell membranes. Although there are numerous methods of lipid extraction available to date, a vast majority of them starts with cell or tissue homogenization. Bead beating has been reported to be one of the most effective and rapid cell & tissue disruption method, increasing lipid extraction efficiency at a lower cost (Kumar et al., 2015).

Here, we present three case studies highlighting the usefulness of the Precellys® homogenizer combined with the Cryolys® cooling unit for sample preparation prior to lipid extraction.

THE PRECELLYS® IN COMBINATION WITH THE RIGHT LYSING KIT HOMOGENIZES ANY TYPE OF SAMPLE RAPIDLY

SUMMARY

DROSOPHILA HOMOGENIZATION FOR LIPID ANALYSIS

Department of Developmental Biology, University of Regensburg, Germany

/ CONTEXT

Our research aims to establish Drosophila as a model for Friedreich´s ataxia. This human neurological disorder is produced by the lack of the mitochondrial protein frataxin. Frataxin depletion results in a mitochondrial dysfunction and metabolic problems. We wanted to study weather reduction of frataxin in Drosophila also induced some metabolic responses such as loss of lipid homeostasis. In this work we have found that ubiquitous and glialtargetted reduction of frataxin expression leads to an increase in fatty acids [1].

[1] J.A. Navarro et al., Altered lipid metabolismin a Drosophila model of Friedreich s ataxia,Human Molecular Genetics, 2010 1–13doi:10.1093/hmg/ddq183.

/ MATERIALS

Precellys®24 homogenizer

Precellys®lysing kit: 03961-1-002 (ceramic beads 2.8mm)

Sample: Drosophila L3 larvae (15) or Drosophila adult heads (80)

Buffer: Water

/ PROTOCOL

Precellys®24: 5500 rpm, 2×25 sec, 10s break

Centrifugation steps: 5000 rpm 60s

Analysis of lipid content: Samples were delipidated according to Bligh and Dyer, 1959 for thin layer chromatography studies and gas chromatography coupled with mass spectrometry (GC/MS) was carried out after FA methyl ester derivatization according to Ecker et al., 2010.

/ RESULTS

Quality and quantity of extracted lipids from Drosophila samples using the Precellys®24 technology was sufficient on the one hand to have reliable and reproducible results from different biological replica (not illustrated), and on the other hand to observe clear differences between control flies and frataxindeficient individuals (Figure1).

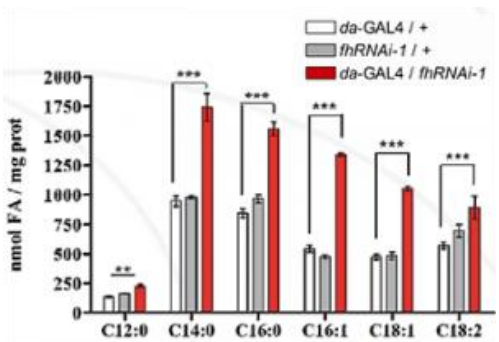


Fig. 1. GC/MS analysis of fatty acids from Drosophila L3 larvae (Myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1) and linoleic acid (C18:2)).

Frataxin deficiency increases the amount of each fatty acid. In conclusion, loss of frataxin affects lipid metabolism and catabolism provoking an accumulation of fatty acids. Moreover, triacylglicerides and other neutral or phospholipids are not so affected.

/ CONCLUSION

/ CUSTOMER

The Precellys®24 provided us a complete fly homogenate containing lipids in the right range of both amount and purity, in order to carry out our experiments. Sample preparation is not only easy but cross contamination free.

LIPID HYDROPEROXIDES (LPO) EXTRACTION FROM TUMOR AND NON-TUMOR RAT TISSUES

Cancer Research Laboratory, University Hospital of Tours, Tours, France

/ CONTEXT

The fundamental and clinical project of our research unit is positioned at the junction of the two fields “cancer and nutrition” with a specialization in lipid biochemistry and breast cancer. Our research unit has described the potential benefit of the clinical use of lipid nutrients in order to increase the efficiency of cancer treatment. Docosahexaenoic acid (DHA) has the potential to increase tumor sensitivity to chemotherapy with no sensitization of normal tissues. This study [1] aimed to explore the mechanism involved in this differential sensitization with a focus on oxidative stress, one of the main determinants involved in DHA enhancement of anthracycline-based chemotherapy.

/ MATERIALS

Precellys®24 homogenizer with Cryolys cooling device to have a constant temperature of 4°C within the homogenization chamber using liquid nitrogen.

Precellys lysing kit: 03961-1-009 (CKmix).

Sample: ~100 mg of frozen tumors, intestine, liver, and heart from rats treated with DHA + epirubicin and from control rats (palm oil, no chemotherapy) .

Buffer: Ice-cold distilled water.

/ PROTOCOL

Precellys setting: 6500 rpm, 3x20sec, 50sec break.

Lipid hydroperoxides (LPO) were extracted and assayed with a lipid hydroperoxides assay kit according to the manufacturer’s instructions (Kit no. 705003, Cayman Chemical Company, Ann Arbor, MI, USA).

/ RESULTS

Overall, our results showed that supplemental DHA during an anthracycline-based chemotherapy selectively increased tumor level of LPO. In fact, at baseline (control group) a similar level of LPO was detected in tumors, liver, heart, and intestine. Supplementing animals with DHA during chemotherapy increased the level of LPO in tumors while no change in LPO level was detected in liver, heart, or intestine (figure 1), even though their enrichment with DHA was larger.
Enzyme activity assays showed a differential change in antioxidant defenses between tumors and other tissues might be a mechanism contributing to the absence of toxicity of DHA supplementation to normal tissues during chemotherapy.

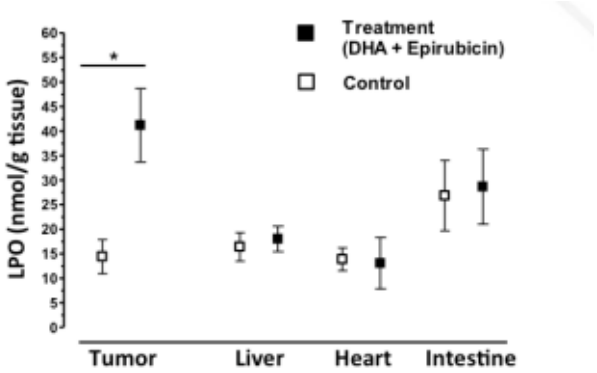


Figure 1: LPO level in tumor and non-tumor tissues at baseline (control rats) and in response to treatment with DHA and epirubicin.

During this study, the Cryolys guaranteed a constant cool temperature within the homogenization chamber.

/ CONCLUSION

The combo Precellys & Cryolys cooling option are suitable and reliable systems to homogenize a large range of rat tissues and tumors to investigate molecular and cellular mechanisms of action of lipids. In our rat model, an efficient and equably homogenization of tissues with the Precellys & the Cryolys was a prerequisite for an optimal subsequent extraction and measure of lipid hydroperoxides in tissues.

HIGH-THROUGHPUT LIPID EXTRACTION FOR THE ANALYSIS OF HUMAN BRAIN LIPIDS

Illawarra Health and Medical Research Institute, University of Wollongong, Australia

/ CONTEXT

Traditional lipid extraction techniques are the bottleneck for modern shotgun lipidomic studies. To overcome this problem, protocol comparisons were made between the traditional Folch extraction (using chloroform and glass-glass homogenization) and a high-throughput method combining methyl-tert-butyl ether (MTBE) with mechanical homogenization (Precellys-24, Bertin Technologies) [1].

[1] Sarah K. Abbott et al. An Improved High-Throughput Lipid Extraction Method for the Analysis of Human Brain Lipids, Lipids ISSN 0024-4201 Volume 48 Number 3 Lipids (2013) 48:307-318 DOI 10.1007/s11745-013-3760-z

/ MATERIALS

Homogenizer: Precellys®24 homogenizer.

Precellys lysing kit: CK14_0.5mL (03961-1-203).

Samples: 10mg pulverized brain aliquot (human occipital cortex) weighted directly into the 0.5mL tube

Buffer: 300µL ice-cold methanol containing internal standards.

/ PROTOCOL

Precellys 24: 6000 rpm, 2×30 sec.

Lipids were extracted using MTBE or chloroform and analyzed using electrospray ionization mass spectrometry (ESI-MS; for phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, ceramide and sphingomyelin) and sterol species analyzed using gas chromatography (GC-MS).

/ RESULTS

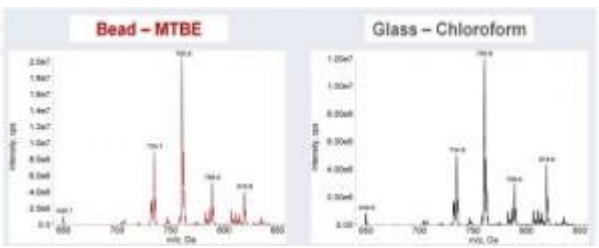


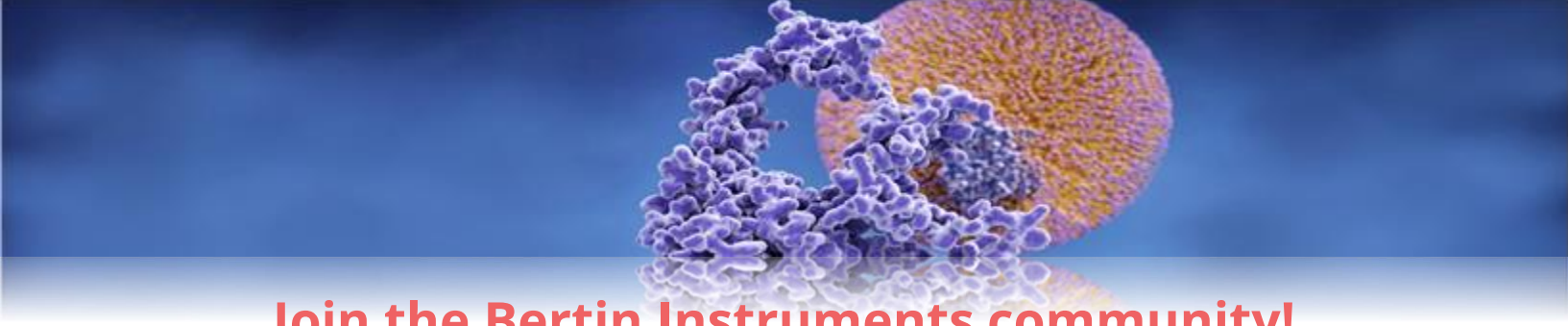
Figure 1: Representative spectra of human occipital cortex comparing Bead – MTBE to Glass – Chloroform (PC head group scan: precursor ion scan m/z 184.1)

This high-throughput Bead-MTBE protocol improves upon traditional lipid extraction methods as it is safer (less carcinogenic / toxic) and much more efficient. The Bead-MTBE protocol is approximately four times quicker than Glass-Chloroform in the homogenization of 24 samples (i.e.1 vs. 4h), with the additional benefit being that tissue aliquots can be weighted directly into the Precellys tubes prior to homogenization (thus reducing double-handling times).

The lower density of MTBE further enhances the lipid extraction procedure (by dissolving lipids in the upper phase) and is also better for the potential incorporation of robotics to further streamline lipidomic studies.

/ CONCLUSION

Lipidomic profiling of human brain tissue using MTBE extraction and mechanical bead homogenization with the Precellys was comparable to traditional extraction techniques (i.e. chloroform extraction with glass-glass homogenization). The Bead-MTBE protocol provides an improved method for lipid extraction, as it is safer and much more efficient.



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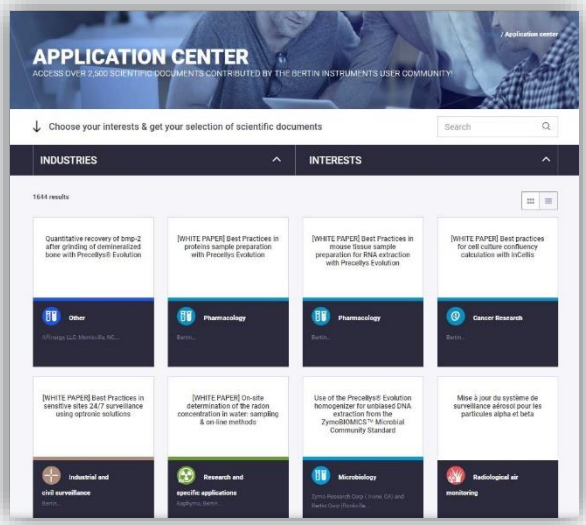

Precellys® Evolution is the most advanced homogenizer gathering high efficiency and versatility for all sample preparation needs:

- **Flexibility:** 24 x 2mL (or 0.5mL), 12 x 7mL, 6 x 15mL and 96 well-plate format
- **Efficiency:** up to 10 000 rpm speed to grind any type of sample
- **Integrity:** protect your molecules with the Cryolys® Evolution cooling unit