مؤسسة مستشفى سرطان الأطفال-مصر
Children's Cancer Hospital Foundation - Egypt
57357

Metabolomics Extraction from Saliva Samples

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1. Time of sample collection must be justified to decrease the interference from the food and drink. It is better to fasten the animals for 1-2hr.
2. Visual inspection must reveal no contaminations, e.g. hair and blood, in the samples.
3. The samples must be stored in a freezer at −80°C until extraction.
4. Equal samples amount must be used during the extraction.
5. Cold extraction buffer with thawing and freezing technique

- Prepare extraction solvent: Methanol: dichloromethane: ethyl acetate 1:2:3.
- Extraction solvent kept in -80°C freezer for at least 2hr.

- 200 µl of the sample is mixed with 900 µl of pre-cooled extraction solvent in an Eppendorf tube.
- Vigorous mixing is done using vortex for 1 minutes.

- The tube is transferred into dry ice or freezer (-80ºC) for 30 min.
- The sample is thawed in an ice bath then it is sonicated for 5 min at 20-30 kHz. (temperature must be not greater than 20ºC)

- The sample is centrifuged at 10,000 for 10 min 4 °C.
- The supernatant is transferred to a new tube.

- The sample is centrifuged at 10,000 for 10 min. The supernatant is transferred to a new tube.
- The supernatant evaporated using vacuum rotary evaporator at 30ºC to approximately 25 µl volume.

- The sample reconstituted in 200µl solvent (water: methanol: acetonitrile 2:1:1).
- Centrifuged at 10,000 for 5min. The supernatant is transferred to analysis tube.

- Analysis, done using 5-25 µl injection volume (depending on the complicity of the sample), is applied to the LC-MS/MS. This will be used for positive and negative mode.

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- Mobile phase depends on the mode:

Positive mode mobile phase	Negative mode phase
DI-Water contains 0.1%FA	5mM ammonium format containing 1% methanol. Then the pH will be adjusted to 8 using ammonium hydroxide.

- Quality control (QC) samples, which are a mixture of equal volume taken from each real sample, also underwent LC-MS/MS analysis for quality assurance of the experiment.
- It is better to spike the sample with internal standards. These internal standard is better that not included in the endogenetic metabolites. You can also use another one depending on experimental design.