مؤسسة مستشفى سرطان الأطفال-مصر
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Metabolomics Extraction from Bacterial Culture Samples

A. Extraction of the bacterial secretory metabolites (Exometabolites)

1. After complete growth, the broth cultures are centrifuged at 10000 rpm for 20 min. 4C, and then filtered through Whatman® filter paper.
2. The filtrates contain the active crude metabolites. The filtrates are extracted by liquid-liquid extraction method
3. Prepare extraction solvent: Methanol: dichloromethane: ethyl acetate, in ratio 1:2:3.
4. Extraction solvent is kept in -80°C freezer for at least 2hr.
5. The sample is mixed with pre-cooled extraction solvent (2:10). For example, 2ml sample +10ml extraction solvent.
6. Vigorous mixing using vortex for 1 minutes.
7. The sample is sonicated for 5 min at 20-30 kHz. (Temperature must be not greater than 20ºC)
8. The sample is centrifuged at 10,000 for 10 min 4 °C.
9. The supernatant is transferred to a new tube.
10. The supernatant is combined and evaporated using vacuum rotary evaporator at 30ºC.
11. The sample is reconstituted in 2ml solvent (water: methanol: acetonitrile 2:1:1).
12. Centrifuge at 10,000 for 5min. The supernatant is transferred to analysis tube.
13. Analysis is don using 10-25 µl injection volume.

- 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.

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B. Extraction of the Exometabolites and Endometabolite

1. Prepare extraction solvent: Methanol: dichloromethane: ethyl acetate 1:2:3.
2. After complete bacterial growth, add the extraction solvent directly for the bacterial growth tube. (before filtration).
3. The sample is mixed with extraction solvent in a ratio (2:10). For example, 2ml sample +10ml extraction solvent.
4. Vigorous mixing using vortex for 2 minutes.
5. The tube is transferred into dry ice or freezer (-80ºC) for 30 min.
6. 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)
7. The sample is centrifuged at 10,000 for 10 min 4 °C.
8. The supernatant is transferred to a new tube.
   (The supernatant contains the active crude metabolites both Exometabolites + endometabolite).
9. The supernatants are combined and evaporated using vacuum rotary evaporator at 30ºC.
10. The sample is reconstituted in 2ml solvent (water: methanol: acetonitrile 2:1:1).
11. Centrifuged at 10,000 for 5min. The supernatant is transferred to analysis tube.
12. Analysis is done using 10-25 µl injection volume (depending on the complexity of the sample).

- 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.