Harvest/Labelling Protocol

General:

* + Keep 12C and 13C treatments as similar as possible!
  + If one works over a day, keep in mind, that cells still grow, HeLas double in 16-22h!, so plan media changes before actual labelling start (4h, 24h) accordingly, and count cells at reasonable times
  + Main Factors in accuracy are: cell count, pipetting of MeOH

Labelling

1. 24h and 4h before experiment start exchange the media, leaving 2 mL of previous media on the plate (to keep some growth factors etc there)
2. From extra plate(s) before starting a block of experiments, count living cells (Trypan blue). ***This is the basis for normalization during data analysis! Reproducibility between plates has to be checked at a previous time point***
3. **Check list before starting**
   1. Cool 50% Methanol with 1 µg/mL Cinnamic Acid
   2. Dry ice for Methanol
   3. Heat Media and wash buffer to 37 °C
   4. Prepare Falcons with 1 mL chloroform (1 per plate)
   5. Prepare Eppis for Media samples (2 per plate)
   6. Unpack Scrapers
   7. Ice for cell scraping
   8. Liquid Nitrogen
   9. Tissue to dry plates
   10. Make sure to have enough tips (especially 5 mL)
   11. Waste bucket
   12. Waste for tips/scrapers and harvested plates
   13. Open all bottles
   14. Remember pipette position
4. **Take 100 µL Media** sample of the plate into 1.5 mL eppi, freeze in liquid nitrogen
   1. Remove Media completely

* 1. Wash with PBS/HEPES (as you would in normal cell culture, you want to remove pre-existing nutrients etc)

* 1. Add **5mL of preincubated labeled Medium** to each plate, *start time*

* 1. Incubate cells in the incubator for a defined time

* 1. Shortly before time ends, **take 100 µL labeled Media sample**, freeze in liquid nitrogen

* 1. Shortly before time is over,remove labeled Media, dry on paper tissue and add **5mL of labeled wash buffer** (HEPES 5mM, NaCl, 140mM, pH 7.4, 500 mL: 4.1 g NaCl + 0.569 g HEPES and e.g. 13C or 12C Glucose, wash buffer contains only the C-source used to label and Methylglyoxal if applicable)

* 1. After swinging the plate
     1. remove the buffer, dry on paper tissue
     2. Put plate on ice, immediately ***at end of labeling time*** add ***exactly*** **5mL of ice cold MeOH**

* 1. Scratch the cells quantitatively from the plate, resuspend with the 5 mL pipet and transfer 5mL into the prelabeled falcons, containing 1mL chloroform **(here the 5 mL are the critical step, *important for quantification!)***

* 1. **Shake vigurously**

* 1. Store in liquid nitrogen either continue with phase separation or store at -80 °C