SMPD1 activity measurement

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

- Substrate buffer
  - 0,1M Sodium acetate buffer pH 5,2 (120µl glacial acetic acid + 79ml 0,1N sodium acetate to 100ml)
  - 0,2%(w/v) synthetic sodium taurocholate
  - 0,02% sodium azide
- HMU-PC substrate
  - 1.32 mM 6-hexadecanoylamino-4-methylumbelliferyl-phosphorylcholine (HMU-PC) in substrate buffer
  - (Mol. Wt: 594.72) [10mg in 12.72ml*heat for short duration at 60 deg-C until clear. Aliquots (1ml each) and sore at -80 deg-C]
- Stop buffer
  - 0.2 M glycine–NaOH buffer (pH 10.7) (910µl 5M NaOH + 25ml of 0.2M glycine~volume to 100ml)
  - 0.2% (w/v) sodiumdodecyl sulphate
  - 0.2% (w/v) Triton X-100

Procedure

1. mix 10µl of homogenate with (standardized quantity of protein 10 µg for fibroblasts) with 20µl of HMU-PC substrate in substrate buffer (1:1 ratio)
2. Incubate for 1 hour
3. Measure fluorescence kinetics for 30 min **(optional!)** **at Ex. 360 Em. 460nm**
4. Add 200µl stop buffer
5. Measure fluorescence **(Endpoint!)**