

# G0244 $\alpha$ -Galactosylceramide Solubilization Protocols

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$\alpha$ -GalCer is a compound that is challenging in terms of solubility. The lack of solubility is an inherent property of the molecule. It was described as insoluble in water, methanol, ethanol or other organic solvents, very slightly soluble in tetrahydrofuran and slightly soluble in pyridine.

Pyridine is often used to solubilize compounds that are not well soluble. However, pyridine as solvent is not suitable for cell culture applications! Below are protocols that worked for researchers in the past.

Different solubilization protocols for  $\alpha$ -Galactosylceramide:

## 1) Tween20 and Sodium chloride $\rightarrow$ Heat $\rightarrow$ Sonicate

- Dissolve  $\alpha$ -GalCer in 0.5% Tween20, 0.9% NaCl solution.
- Heat to 85°C until solution turns cloudy.
- Take out of water bath and keep at RT.
- Solution will become clear quickly.
- In case some particles are left, sonicate (important: sonication has to be performed in glass vial, it does not work in plastic Eppendorf tube!) and filter afterwards with 2  $\mu$ m filter.

## 2) Tween20 and PBS $\rightarrow$ Heat $\rightarrow$ Sonicate

- Dissolve  $\alpha$ -GalCer in 0.2mg/ml PBS containing 0.5% Tween20 (warm up and sonicate 2 hrs. at 37°C).
- The solution can be more a suspension than a clear solution.
- In case some particles are left, warm up and sonicate (important: sonication has to be performed in glass vial, it does not work in plastic Eppendorf tube!).
- For sonication no interval is recommended, just use a conventional water bath sonicator at standard amplitude.

## 3) DMSO $\rightarrow$ Heat $\rightarrow$ Sonicate

- Dissolve  $\alpha$ -GalCer in DMSO at the concentration of 1mg/ml with heating at 80°C for several minutes.
- Sonication for 2 h.
- The solution of 1mg/ml in DMSO can be further diluted in PBS.

## 4) 2 Steps: Chloroform:Methanol $\rightarrow$ Evaporation $\rightarrow$ DMSO, PBS or Tween20 $\rightarrow$ Heat $\rightarrow$ Sonicate

- Dissolve  $\alpha$ -GalCer in a mixture of chloroform:methanol (2:1).
- Aliquot into reasonable aliquots for the experiment.
- Evaporate the solvent using a gentle stream of nitrogen so that you have a thin, dry film of material at the bottom of the vial.
- Add either DMSO or PBS + 0.5% Tween20 to achieve your final concentration.
- Some heating and sonication might be necessary.
- The thin film of compound generated by the evaporation step allows greater access of the compound to the solvent and better dissolution.

## 5) 5.6% sucrose, 0.75% L-histidine and 0.5% Tween20 $\rightarrow$ Heat $\rightarrow$ Sonicate

- Dissolve  $\alpha$ -GalCer in 5.6% sucrose, 0.75% L-histidine and 0.5% Tween20
- Heat at 80°C for several minutes and sonicate.

IMPORTANT:  $\alpha$ -GalCer can be stored in solution in glass vials at 4°C or -20°C, and is stable for ~3 months also when dissolved!

