# [Protocol 1.03.1] Reconstitution, aliquoting and use of Y26732 ROCK inhibitor

Author: Jeong-Eun Lee
No tags associated

Created: 20.12.2021 18:56
Last modified: 11.08.2022 14:19

No custom dates added

Protocol 1.03.1 Reconstitution, aliquoting and use of Y26732 ROCK inhibitor
Version: 1.0 (20.12.2021)

**Media and Reagents:**
ROCK inhibitor (Y-27632; Stem Cell Technologies, 72305)
Distilled sterile water

Alternatively in DMSO, ready to use:
ROCK inhibitor (Y-27632 2HCl; Selleck Chemicals SEL-S1049-10mM pre-solved)

**Materials and Equipment:**
Sterile syringe filter (low protein-binding!)
0,5 ml Eppis

**1. Introduction and Purpose**
This protocol describes the storage of Y-27632 ROCK inhibitor and the preparation of media containing Y-27632 ROCK inhibitor. Y-27632 is a ≥ 95% pure synthetic compound with a molecular weight of about 325 g/mol. It is light-sensitive and hygroscopic.

**2. Reconstitution and aliquoting**
Centrifuge the vial (Stem Cell Technologies, 72305) prior to opening.Reconstitute in sterile water.

| ReconstituteY-27632 | in sterile water | Concentration of solution |
|---|---|---|
| 10 mg | 5,912 ml | 5 mM |

Filter sterile using a low protein binding filter and prepare aliquots of 200 µl. Store these at -20°C.

Alternatively, 10 mM ROCKi in DMSO, ready to use (Selleck Chemicals):
Prepare aliquots of 50 -100 µl and store these at -20°C or at -80°C for long-term storage up to two years.

The location of aliquots: TBA

**3. Preparation of medium containing ROCK inhibitor**
Thawed aliquots of Y-27632 ROCK inhibitor can be stored at 4°C for up to two weeks.
The final concentration of Y-27632 ROCK inhibitor in the culture medium should be 10 µM. Therefore, the stocks will be used 500x (5mM) or 1000x (10mM) diluted.
Following table shows the apposite volume of Y-27632 ROCK inhibitor for different volumes of medium.

| VolumeMedium[ml] | Volume ROCKi 5mM (Stem Cell Tech) [µl] | Volume ROCKi 10mM (Selleck Chem)[µl] |
|---|---|---|
| 5 | 10 | 5 |
| 12 | 24 | 12 |
| 50 | 100 | 50 |
| 500 | 1000 | 500 |

**Relevant applicable documents:**
Protocol 1.03.0 Passaging of iPSC into single cells using TrypLE/Accutase
Protocol 1.04.0 Sorting of differentiated cells using MACS