

January 2014

# Y27632 treatment Handbook



NSF/EDEN Grant number IOS # 0955517

## Required Equipment

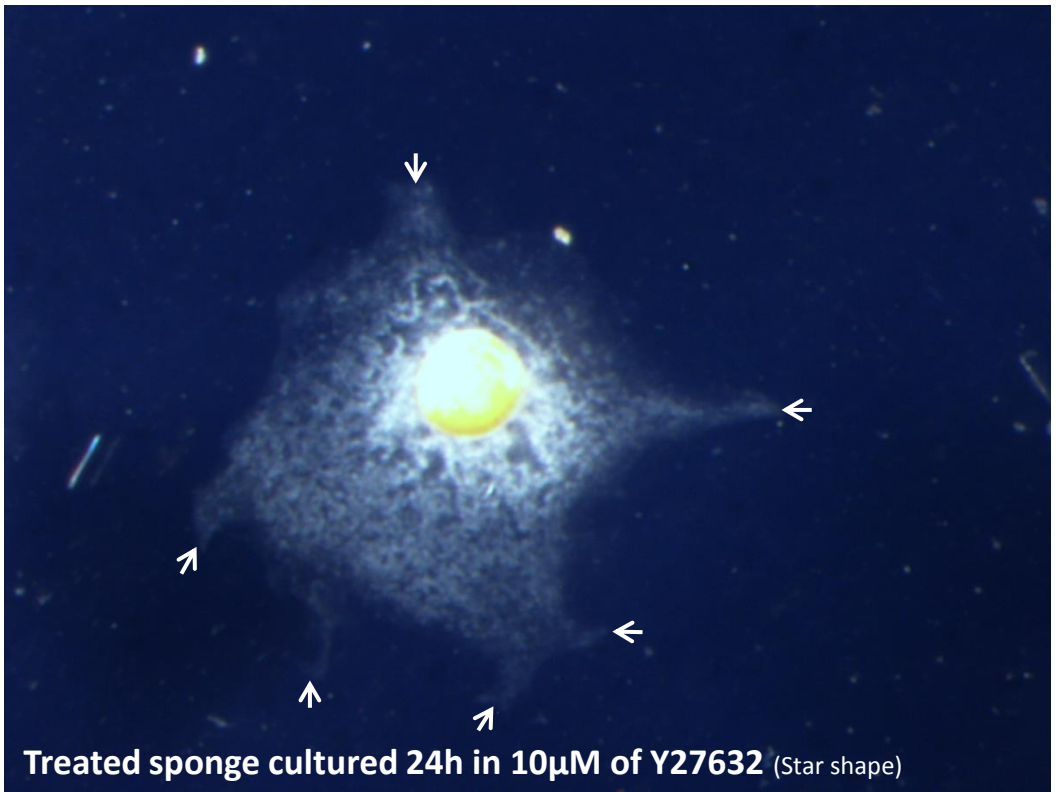
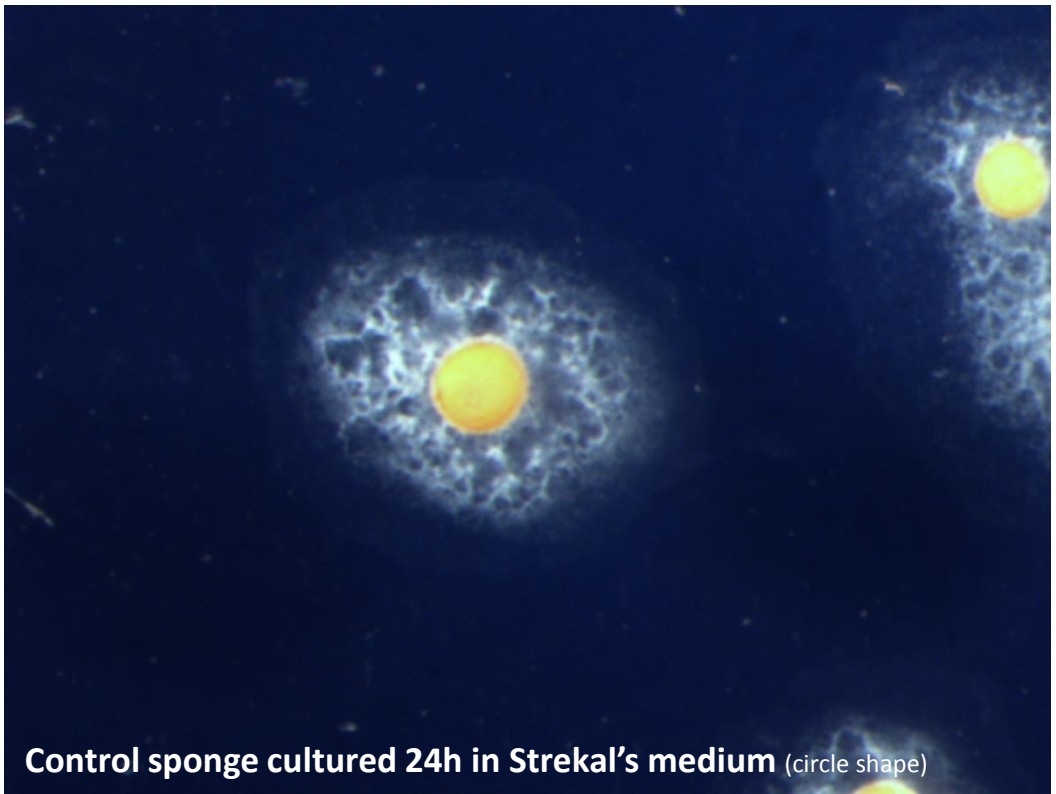
<b>Sample</b>	
Attached gemmules in a 12 wells plate	
<b>Solutions</b>	
Strekal's Medium	1mL/well/day
Y27632 stock solution (2,5mM)	4μL/well/day

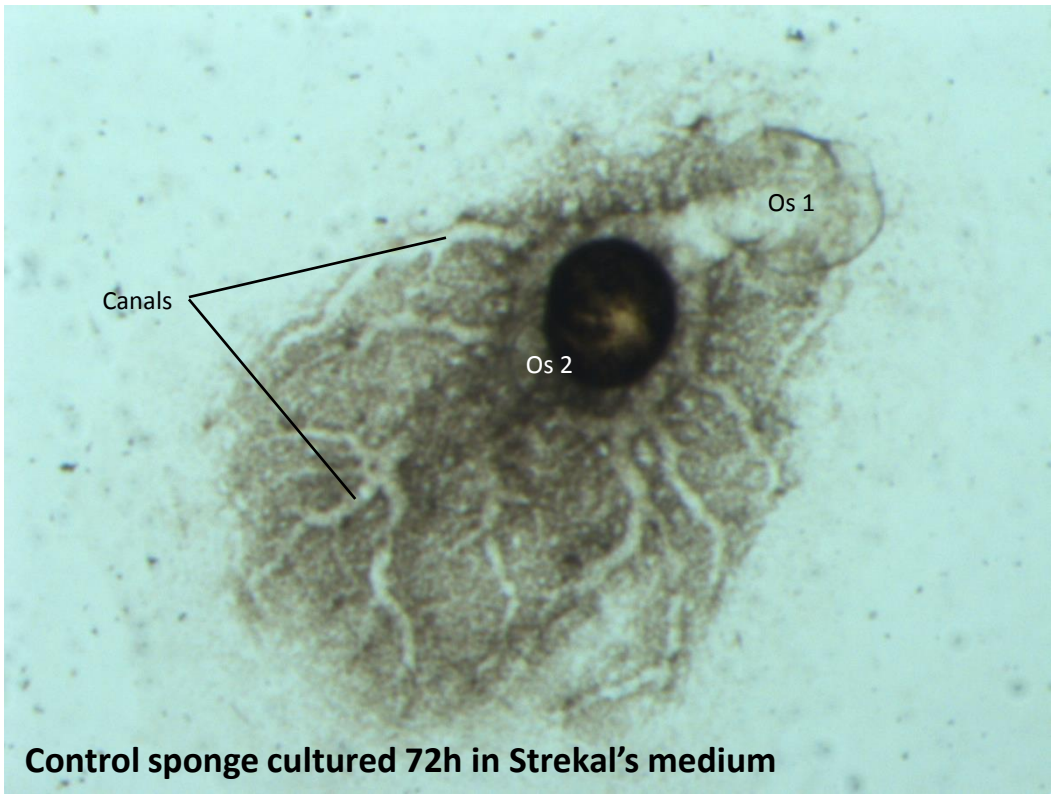
## Y27632 stock solution

Y27632 is a specific ROCK inhibitor. 1 mg of Y27632 . dihydrochloride (MW: 247.3 . 73.0) was purchased at Enzo® Life Science and diluted in 1.25mL of distilled water giving a 2.5mM stock solution. Aliquots were made to avoid thawing and freezing them several times. They were covered with aluminum foil to prevent them from the light and store at -20°C.

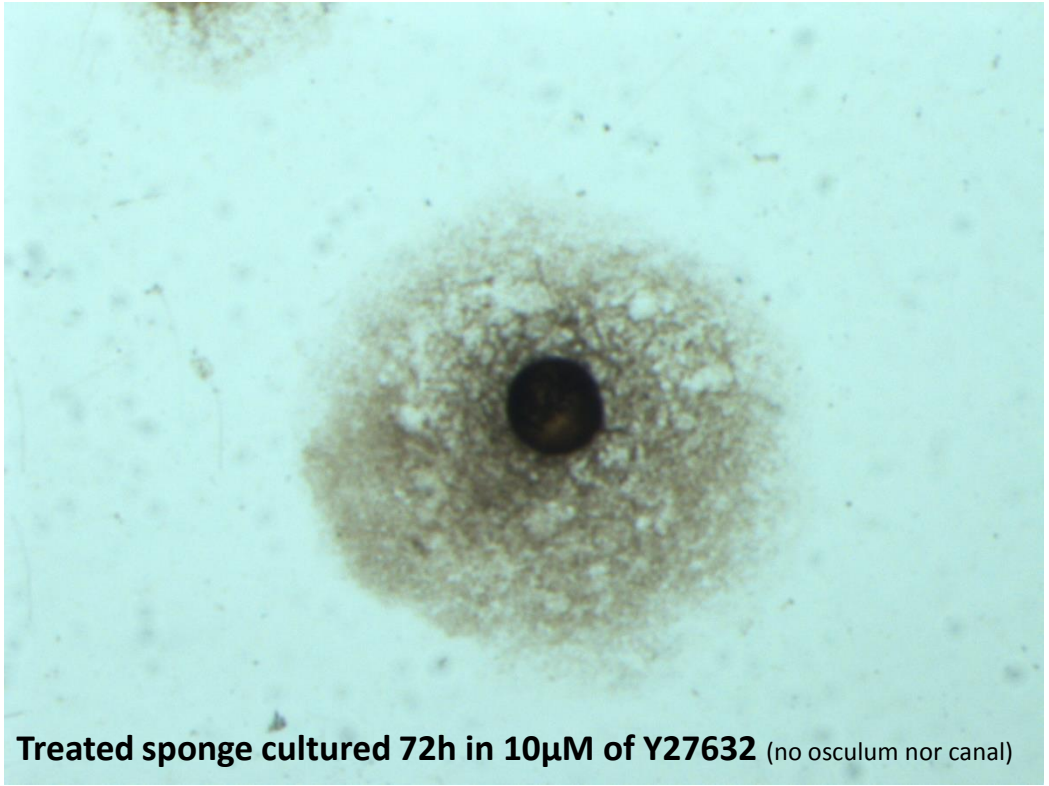
## Drug treatment

- 1. Check that gemmules are at the desire stage of development and take pictures of them.**  
Usually just after attachment.
- 2. Dilute Y27632 stock solution in new Strekal's Medium.**  
For example 4μL in 1mL for a final concentration of 10μM.
- 3. Well by well, quickly but gently replace de medium with the new medium containing the drug.**
- 4. Change all the solution every 24h.**  
Also in control wells.
- 5. Take pictures as often than required.**





**Control sponge cultured 72h in Strekal's medium**



**Treated sponge cultured 72h in 10µM of Y27632 (no osculum nor canal)**

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