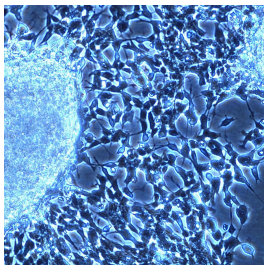
Feb 06, 2019

Making Differentiation Media for SH-SY5Y



DOI
https://dx.doi.org/10.17504/protocols.io.xuufnww

Kenneth Schackart[1]

[1]University of Arizona

Yoon Lab

Kenneth Schackart
University of Arizona

DOI: https://dx.doi.org/10.17504/protocols.io.xuufnww

Protocol Citation: Kenneth Schackart 2019. Making Differentiation Media for SH-SY5Y. **protocols.io** https://dx.doi.org/10.17504/protocols.io.xuufnww

Protocol status: In development
We are still developing and optimizing this protocol

Created: February 06, 2019

Last Modified: February 06, 2019

Protocol Integer ID: 20084

Keywords: differentiation media for sh, making differentiation media, differentiation medium for sh, differentiation medium, sy5y

Abstract

This describes how to make differentiation medium for SH-SY5Y using all-*trans*-retinoic acid.

Materials

- all-*trans*-retinoic acid
- B-27 Supplement
- L-glutamine
- Neurobasal Medium (NBM)
- 100% Ethanol
- Autoclaved deionized water (ADIW)

Make ATRA Stock Solution

1 In a 15 mL centrifuge tube, add 14.25 mL ethanol and 0.75 mL autoclaved deionized water. This will make a 95% ethanol solution.

2 Transfer 3330 µL of 95% EtOH solution to another 15 mL centrifuge tube.

3 Weigh out 5 mg ATRA.

> Note
>
> ATRA is light sensitive, so do this step with the lights off.

4 Add the 5 mg ATRA to the 3330 µL 95% EtOH solution. This makes a 5 mM stock ATRA solution.

5 Vortex mix the solution.

6 This solution is used at a rate of 100 µL stock solution per 50 mL differentiation media. The final concentration should be 10 µM.

> Note
>
> We most recently made 10 aliquots, each with 62 µL. This is to make 10 30 mL media batches, each using 60 µL.

7 Stock solution can be stored for up to 6 weeks at 4 °C.

Make l-Glutamine Stock Solution

8 Weigh out 175 mg l-glutamine.

9 Aliquot 15 mL NBM into a 15 mL centrifuge tube.

10 Add the l-glutamine to the NBM.

11 Vortex mix the solution.

12 Aliquot the solution into 1.5 mL aliquots.
This solution is 20x. The concentration is 80 mM and the final desired concentration is 4 mM.

> Note
>
> This protocol makes 10 aliquots of 1.5 mL. Each stock aliquot makes 30 mL differentiation medium.

13 Store aliquots at -20 °C.

Aliquot B-27 Supplement

14 Aliquot 600 µL batches of 50x B-27 supplement.

15 Each aliquot will make 30 mL differentiation media.

16 Store aliquots at -20 °C .

Making the media

17

> Note
>
> Do not mix the media until you are ready to change the media in a flask. It degrades quickly.

Warm 30 mL NBM in water bath.

18 Thaw one 50x B-27 supplement aliquot, and one 20x l-glutamine supplement.

19 In biosafety cabinet, combine the following in a 50 mL tube

- 29.34 mL Nuerobasal Medium
- 600 µL 50x B-27 Supplement
- 1.5 mL 20x l-glutamine Stock Solution
- 60 µL 500x All-*trans*-retinoic Acid Stock Solution

20 Sterile filter the differentiation media.

21 Use immediately.