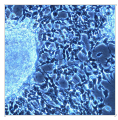

Making Differentiation Media for SH-SY5Y

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ABSTRACT

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

PROTOCOL STATUS

In development

We are still developing and optimizing this protocol

MATERIALS TEXT

- 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.