Jan 21, 2026

Differentiation of SH-SY5Y Cells in a 96-Well Plate Format

DOI
https://dx.doi.org/10.17504/protocols.io.yxmvm13m5v3p/v1

Qianying He[1]

[1]Department of Biosystems Engineering, University of Arizona, Tucson, Arizona, USA

Q Qianying He

DOI: https://dx.doi.org/10.17504/protocols.io.yxmvm13m5v3p/v1

Protocol Citation: Qianying He 2026. Differentiation of SH-SY5Y Cells in a 96-Well Plate Format. **protocols.io** https://dx.doi.org/10.17504/protocols.io.yxmvm13m5v3p/v1

Protocol status: Working
We used this protocol and it's working

Created: January 17, 2026

Last Modified: January 21, 2026

Protocol Integer ID: 238836

Keywords: sy5y neuronal differentiation, sy5y neuroblastoma cell, sy5y cell, reproducible neuronal differentiation, neuronal differentiation, model for neuronal differentiation, format culture vessel, neuroblastoma cell, standardized differentiation protocol, well plate format sh, established differentiation protocol, well plate format, differentiation protocol, differentiation of sh, throughput assay, neurodegenerative disease research, differentiating sh, differentiation

Abstract

SH-SY5Y neuroblastoma cells are widely used as an in vitro model for neuronal differentiation and neurodegenerative disease research. However, most established differentiation protocols are optimized for large-format culture vessels, such as 35 mm dishes, and do not translate directly to high-throughput applications. Therefore, a standardized differentiation protocol specifically adapted for 96-well plate formats has not been well established.

Here, we describe a step-by-step protocol for differentiating SH-SY5Y cells in a 96-well plate format. This protocol is adapted from a commonly used 35 mm dish–based method and has been systematically scaled to accommodate reduced working volumes, well surface area, and multichannel pipetting. Key modifications include optimized seeding density, media volumes, partial media changes, and plate-handling practices designed to minimize edge effects and cell loss during long-term differentiation.

This 96-well–adapted protocol enables reproducible neuronal differentiation while maintaining compatibility with drug screening and other high-throughput assays. It provides a practical and accessible framework for laboratories seeking to perform SH-SY5Y neuronal differentiation in a 96-well plate format commonly used for screening applications.

Materials

Growth Media Components:
- EMEM (Fisher 50-238-2632)
- 15% hiFBS (Gibco A5209501)
- 1x Pen/Strep (Gibco 15070063)
- 2 mM Glutamine (Gibco 25030081)

Differentiation Media #1 Components:
- EMEM (Fisher 50-238-2632)
- 2.5% hiFBS (Gibco A5209501)
- 1x Pen/Strep (Gibco 15070063)
- 2 mM Glutamine (Gibco 25030081)
- 10 µM RA (Sigma R2625-50MG)

Differentiation Media #2 Components:
- EMEM (Fisher 50-238-2632)
- 1% hiFBS (Gibco A5209501)
- 1x Pen/Strep (Gibco 15070063)
- 2 mM Glutamine (Gibco 25030081)
- 10 µM RA (Sigma R2625-50MG)

Differentiation Media #3 Components:
- Neurobasal plus medium (Gibco A3582901)
- 1x B-27 plus (Gibco A3582801)
- 20 mM KCl (Invitrogen AM9640G)
- 1x Pen/Strep (Gibco 15070063)
- 2 mM GlutamaxI (Thermo fisher 35050061)
- 50 ng/ml BDNF (Sigma B3795-5UG)
- 2 mM dibutyryl cyclic AMP (db-cAMP) (Sigma D0260-5MG)
- 10 µM RA (Sigma R2625-50MG)

Safety warnings

Note: We do not recommend splitting SH-SY5Y maintenance cultures beyond 1:5, as excessive dilution may cause cell death due to low confluency.

Because RA is unstable, it is recommended to prepare media in ~50 mL batches. RA is light sensitive; prepare and handle under low-light conditions.

Before start

- Prepare Growth Media in advance.
- Thaw a frozen vial of SH-SY5Y cells in a 37 °C water bath for approximately 2 minutes.
- Transfer cells to 9 mL Growth Media in a 15 mL conical tube and centrifuge at 1,000 × g for 5 minutes.
- Aspirate the supernatant and resuspend cells in 10 mL Growth Media.
- Plate cells into a 96-well tissue culture–treated plate at a density expected to reach approximately 80% confluency before initiating differentiation.
- To allow time to assess cell health, cells are typically plated at ~60–80% confluency, depending on vial cell count.
- Do not initiate differentiation the day after thawing.
- Replace media the following day to remove dead cells and debris.

Passage of SH-SY5Y Cells (Maintenance)

1 To passage cells from a T-75 flask, aspirate media and rinse once with approximately 10 mL 1× PBS (Gibco 10010023).
Note: We do not recommend splitting SH-SY5Y maintenance cultures beyond 1:5, as excessive dilution may cause cell death due to low confluency.

2 Aspirate PBS and add 2.5 mL 0.05% Trypsin-EDTA (Gibco 25200056).

3 Incubate at 37 °C for 2–3 minutes, gently tapping the flask to facilitate detachment.

4 Stop trypsinization by adding 10 mL Growth Media and triturate 1–2 times to obtain a single-cell suspension.

5 Centrifuge at 1,000 × g for 5 minutes, aspirate the supernatant, and resuspend the cell pellet in 5 mL Growth Media.

6 Dilute cells 1:3 to 1:5 in a total volume of 20 mL for routine maintenance in a T-75 flask, or proceed to plating for differentiation (see Day 0).

Thawing and Expansion of Undifferentiated SH-SY5Y Cells

7 Prepare Growth Media in advance.

8 Thaw a frozen vial of SH-SY5Y cells in a 37 °C water bath for approximately 2 minutes.

9 Transfer cells to 9 mL Growth Media in a 15 mL conical tube and centrifuge at 1,000 × g for 5 minutes.

10 Aspirate the supernatant and resuspend cells in 10 mL Growth Media.

11 Plate cells into a 96-well tissue culture–treated plate at a density expected to reach approximately 80% confluency before initiating differentiation.

12 To allow time to assess cell health, cells are typically plated at ~60–80% confluency, depending on vial cell count.
Do not initiate differentiation the day after thawing.

13 Replace media the following day to remove dead cells and debris.

Day 0: Plating Cells for Differentiation (96-Well Format)

14 Ensure SH-SY5Y cells are healthy and have reached approximately 80% confluency.

15 Aspirate spent media and replace with Growth Media if needed to standardize volume.

16 Plate 100 µL per well into a 96-well tissue culture–treated plate.

17 Incubate overnight at 37 °C, 5% CO_2 to allow firm attachment.

18 Replace media the following day to remove dead cells.
Note: To minimize edge effects, outer wells may be filled with sterile PBS or unused medium and excluded from downstream analysis.

Day 1: Media Change (Differentiation Media #1)

19 Warm Differentiation Media #1 to 37 °C.

20 Immediately before use, add Retinoic Acid (RA) to the final concentration specified in Table 1.
Because RA is unstable, it is recommended to prepare media in ~50 mL batches. RA is light sensitive; prepare and handle under low-light conditions.

21 Gently aspirate spent media from each well.

22 Add 100 µL Differentiation Media #1 with RA to each well.

23 Return the plate to the incubator.

Day 3 and Day 5: Media Change (Differentiation Media #1)

24 Repeat Day 1 media change, replacing old media with 100 µL fresh Differentiation Media #1 with RA per well.

Day 7: Split Cells 1:1 (96-Well Adaptation)

25 Prepare and equilibrate Differentiation Media #1 with RA as described above.

26 Aspirate old media from each well.

27 Using a multichannel pipette, add 10 µL pre-warmed 0.05% Trypsin-EDTA to each well.

28 Incubate at 37 °C for 2–3 minutes, monitoring under a microscope until cells detach.

29 Stop trypsinization by adding 90 µL Differentiation Media #1 with RA to each well.

30 Gently triturate 1–2 times using a multichannel pipette.

31 Transfer 100 µL cell suspension into a new 96-well plate containing 100 µL Differentiation Media #1 with RA per well to achieve a 1:1 split.

32 Return the plate to the incubator.

Day 8: Media Change (Differentiation Media #2)

33 Warm and equilibrate Differentiation Media #2.

34 Add RA immediately before use.

35 Carefully aspirate old media.

36 Add 100 µL Differentiation Media #2 with RA per well.

37 Return the plate to the incubator, avoiding prolonged exposure of cells to air.

Day 8: Preparation of Poly-D-Lysine–Coated 96-Well Plates

38 Thaw poly-D-lysine (1 mg/mL; Sigma) on ice and dilute 1:20 in sterile ddH_2O.

39 Add 50 µL diluted poly-D-lysine to each well of a new 96-well plate, ensuring full surface coverage.

40 Incubate at 37 °C, 5% CO_2 for 1 hour.

41 Aspirate the solution and allow wells to air-dry in a biosafety cabinet for approximately 1 hour.

42 Seal plates with parafilm and store at 4 °C for up to 2 months.

Day 9: Transfer Cells onto Poly-D-Lysine–Coated Plates (1:1)

43 Warm and equilibrate Differentiation Media #2 with RA.
Aspirate media from differentiated cultures.
Add 10 µL Trypsin-EDTA per well and incubate at room temperature for 1–2 minutes, monitoring detachment.
Quench trypsin by adding 90 µL Differentiation Media #2.
Gently triturate 1–2 times.
Transfer 100 µL cell suspension to poly-D-lysine–coated wells containing 100 µL Differentiation Media #2 with RA.
Return the plate to the incubator.

Day 11, Day 14, and Day 17: Media Change (Differentiation Media #3)

44 Warm and equilibrate Differentiation Media #3.
Add RA immediately before use.
Carefully aspirate old media.
Add 100 µL Differentiation Media #3 with RA per well.
Return the plate to the incubator.

Day 18: Neuronal Cultures Ready for Use

45 SH-SY5Y cells should display mature neuronal morphology.
Replace media with fresh Differentiation Media #3 with RA every 3 days to maintain neuron health.
Cultures are typically stable for up to 14 days following terminal differentiation.

Day 19: Neuronal Cultures for High-Throughput Screening

46 Neuronal cultures may be transitioned to screening-specific neuronal media.
During media changes, pre-wash pipette tips with neuronal media before use and consistently use pre-washed tips to minimize cell stress and variability.