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ab242289
Stem Cell Colony Formation Assay Kit

View Stem Cell Colony Formation Assay Kit datasheet:
www.abcam.com/ab242289

For the measurement of colony formation.

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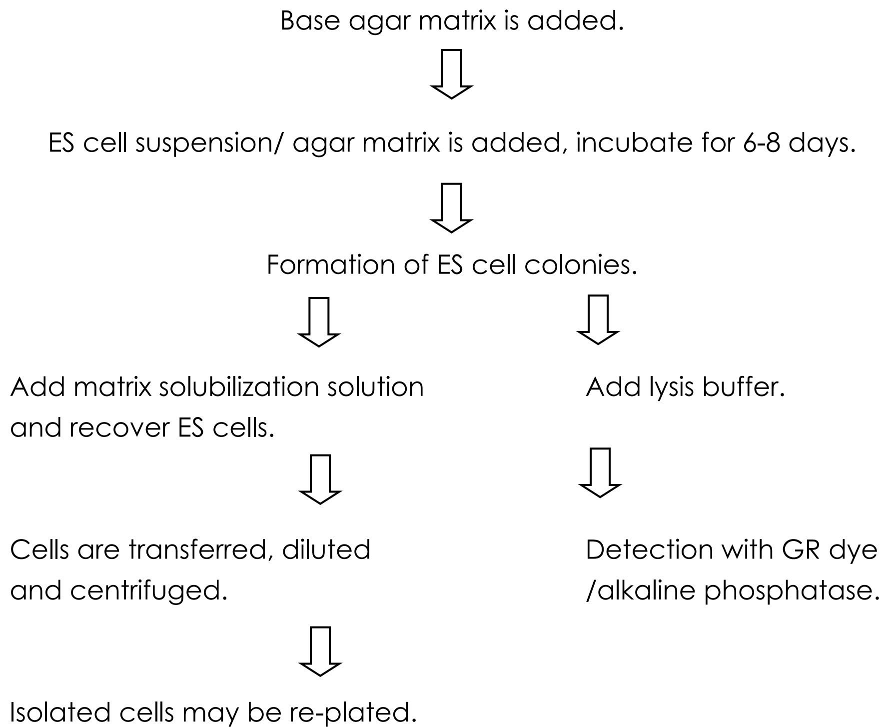
1. Overview

The Stem Cell Colony Formation Assay Kit (ab242289) provides a stringent, anchorage- independent model for measuring stem cell colony formation and allows for cell recovery. Each kit provides sufficient quantities to perform 96 tests in a microtiter plate. The assay may be modified for use in other plate formats.

This kit does not involve subjective manual counting of colonies or require a 3-4 week incubation period. Instead cells are incubated only 6-8 days in a proprietary semisolid agar media before being solubilized, lysed and detected by the GR Dye in a fluorescence plate reader or assayed for alkaline phosphatase activity colorimetrically.

Alternatively, viable colony-forming cells (CFCs) can be easily recovered for further culturing and testing. This format provides a quantitative, high-throughput method to accurately measure colony formation, while the short incubation time makes it possible to assay transiently transfected cells.

2. Protocol Summary



3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at 4°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
10X Agar Matrix Solution	10 mL	4°C
Matrix Diluent	4 mL	4°C
5X DMEM Solution	1.5 mL	4°C
10X Matrix Solubilization Solution	1.8 mL	4°C
GR Dye	75 µL	4°C
Lysis Buffer	20 mL	4°C
AP Activity Assay Substrate	5 mL	4°C
AP Stop Solution	20 mL	4°C
AP Activity Assay Standard	1 mL	4°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Stem cells and culture medium
- Light microscope
- 96-well fluorometer
- 96-well sterile microplate (flat bottom)
- 37°C and boiling water baths
- 37°C incubator, 5% CO_2 atmosphere

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.
- Any components not listed here are ready to use as supplied.

6.1 2X DMEM/20% FBS Medium:

In a sterile tube, dilute the provided 5X DMEM in sterile cell culture grade water to 2X containing 20% FBS. For example, to prepare a 5 mL solution, add 2 mL of 5X DMEM, 1 mL of FBS and 2 mL of sterile cell culture grade water. Additional supplements (e.g. LIF, bFGF, SCF) may also be added at a 2X formulation. Sterile filter the 2X media to 0.2 µm.

6.2 1X Matrix Solubilization Solution:

Dilute the provided 10X stock 1:10 in sterile cell culture grade water. Sterile filter the 1X solution to 0.2 µm.

6.3 10X Agar Matrix Solution:

Heat the Agar Matrix Solution bottle to 90-95°C in a water bath for 30 minutes, or until agar matrix liquefies (microwaving is optional). Transfer the bottle to a 37°C water bath for 20 minutes and maintain until needed.

7. Cell Dose Curve (Optional)

7.1 Heat the 10X Agar Matrix Solution to 90-95°C in a water bath for 30 minutes, or until agar matrix liquefies (microwaving is optional). Transfer the bottle to a 37°C water bath for 20 minutes and maintain until needed.

7.2 Warm the 2X DMEM/20% FBS medium and Matrix Diluent to 37°C in a water bath. Allow at least 30 minutes for the temperature to equilibrate.

7.3 Harvest and resuspend cells in culture medium at 1-5 x 10^6 cells/mL.

7.4 Prepare a serial 2-fold dilution in culture medium, including a blank without cells.

7.5 Transfer 50 µL of each dilution to a 96-well plate.

7.6 According to the table below, prepare the desired volume of Cell Dose Curve Solution in the following sequence:

- In a sterile tube, add the appropriate volume of 2X DMEM/20% FBS medium.
- Next, add the corresponding volume of sterile water. Mix well.
- Next, add the corresponding volume of ™ Matrix Diluent. Mix well.
- Finally, add the corresponding volume of 10X ™ Agar Matrix Solution. Mix well.

Δ Note: The Matrix Diluent and 10X Agar Matrix Solution are slightly viscous; care should be taken in accurately pipetting the appropriate volumes.

2X DMEM/20% FBS Medium (mL)	Sterile Water (mL)	Matrix Diluent (mL)	10X Agar Matrix Solution (mL)	Total Volume of Cell Dose Curve Solution (mL)
1.25	0.45	0.55	0.25	**2.5**
0.625	0.225	0.275	0.125	**1.25**

7.7 Immediately dispense 125 µL of Cell Dose Curve Solution into the wells of the 96-well plate, already containing the cell serial dilution (from step 7.5).

7.8 Add 125 µL of Lysis Buffer to each well.

7.9 Pipette the entire volume of the well 15-20 times to mix thoroughly, lysing the cells completely.

7.10 Transfer 50 µL of the mixture to a 96-well plate suitable for fluorescence measurement.

7.11 Prepare sufficient PBS/GR dye solution for all samples by diluting the dye 1:200 in PBS (for example, add 5 µL dye to 995 µL of PBS).

7.12 Add 100 µL of PBS/GR dye solution to each well (already containing 50 µL of solution). Incubate the plate at room temperature for 30 minutes.

7.13 Pipette each well 7-10 times to ensure a homogeneous mixture.

7.14 Read the plate in a 96-well fluorometer using a 485/520 nm filter set.

8. Assay Protocol

8.1 The following assay protocol is written for a 96-well format. Refer to the below table for the appropriate dispensing volumes of other plate formats.

Culture Dish (µL/well)	96-well	48-well	24-well	12-well	6-well
Base Agar Matrix Layer	50	100	250	500	1000
Cell Suspension/Agar Matrix Layer	75	150	375	750	1500
Culture Media	50	100	250	500	1000
Lysis Buffer	125	250	625	1250	2500
1X Matrix Solubilization Solution	125	250	625	1250	2500

9. Preparation of Base Agar Matrix Layer

9.1 Heat the 10X Agar Matrix Solution to 90-95°C in a water bath for 30 minutes, or until agar matrix liquefies (microwaving is optional). Transfer the bottle to a 37°C water bath for 20 minutes and maintain until needed.

9.2 Warm the 2X DMEM/20% FBS medium to 37°C in a water bath. Allow at least 30 minutes for the temperature to equilibrate.

9.3 According to the table below, prepare the desired volume of Base Agar Matrix Layer in the following sequence:

- In a sterile tube, add the appropriate volume of 2X DMEM/20% FBS medium.
- Next, add the corresponding volume of sterile water. Mix well.
- Finally, add the corresponding volume of 10X Agar Matrix Solution. Mix well.

Δ Note: The 10X Agar Matrix Solution is slightly viscous; care should be taken in accurately pipetting the appropriate volume.

2X DMEM/20% FBS Medium (mL)	Sterile Water (mL)	10X Agar Matrix Solution (mL)	Total Volume of Base Agar Matrix Layer (mL)	# of Tests in 96- well Plate (50 µL/test)
2.5	2	0.5	5	100
1.25	1	0.25	2.5	50
0.5	0.4	0.1	1	20

9.4 After mixing, maintain the Base Agar Matrix Layer at 37°C to avoid gelation.

9.5 Dispense 50 µL of Base Agar Matrix Layer into each well of a 96-well sterile flat-bottom microplate (samples should be assayed in triplicate). Gently tap the plate a few times to ensure the Base Agar Matrix Layer evenly covers the wells.

Δ Note: Work quickly with the layer to avoid gelation. Also, try to avoid adding air bubbles to the well.

Δ **Note:** To avoid fast and uneven evaporation that leads to aberrant results, we suggest not using the wells on the plate edge or filling the edge wells with medium to reduce evaporation.

9.6 Transfer the plate to 4°C for 30 minutes to allow the Base Agar Matrix Layer to solidify.

9.7 Prior to adding the Cell Suspension/Agar Matrix Layer (Section 10), allow the plate to warm to room temperature for 30 minutes.

10. Addition of Cell Suspension/Agar Matrix Layer

10.1 Heat the 10X Agar Matrix Solution to 90-95°C in a water bath for 30 minutes, or until agar matrix liquefies (microwaving is optional). Transfer the bottle to a 37°C water bath for 20 minutes and maintain until needed.

10.2 Warm the 2X DMEM/20% FBS medium and Matrix Diluent to 37°C in a water bath. Allow at least 30 minutes for the temperature to equilibrate.

10.3 Harvest and resuspend cells in culture medium at 0.1-1 x 10^6 cells/mL. Keep the cell suspension warm in a 37°C water bath.

10.4 According to the table below, prepare the desired volume of Cell Suspension/Agar Matrix Layer in the following sequence:

- In a sterile tube, add the appropriate volume of 2X DMEM/20% FBS medium.
- Next, add the corresponding volume of Matrix Diluent. Mix well.
- Next, add the corresponding volume of 10X Agar Matrix Solution. Mix well.
- Finally, add the corresponding volume of cell suspension. Mix well.

Δ Note: The Matrix Diluent and 10X Agar Matrix Solution are slightly viscous; care should be taken in accurately pipetting the appropriate volumes.

2X DMEM/20% FBS Medium (mL)	Matrix Diluent (mL)	10X Agar Matrix Solution (mL)	Cell Suspension (mL)	Total Volume of Cell Suspension / Agar Matrix Layer (mL)	**# of Tests in 96-well Plate (75 µL/test)**
3.5	2.75	0.75	0.5	7.5	**100**
1.75	1.375	1.375	0.25	3.75	**50**
0.875	0.688	0.188	0.125	1.875	**25**

10.5 After mixing, incubate the Cell Suspension/Agar Matrix Layer at room temperature for 5 minutes.

10.6 Immediately dispense 75 µL of Cell Suspension/Agar Matrix Layer into each well of the 96- well plate, already containing the Base Agar Matrix Layer (Section 9).

Δ Note: Work quickly with the layer to avoid gelation, but gently pipette as not to disrupt the base layer integrity. Also, try to avoid adding air bubbles to the well.

Δ Note: Always include negative control wells that contain no cells in the Cell Suspension/Agar Matrix Layer.

10.7 Transfer the plate to 4°C for 20 minutes to allow the Cell Suspension/Agar Matrix Layer to solidify.

10.8 Allow the plate to warm to room temperature for 30 minutes.

10.9 Add 50 µL of culture medium containing cell growth activator(s) or inhibitor(s) to each well.

10.10 Incubate the cells for 6-8 days at 37°C and 5% CO_2. Examine the colony formation under a light microscope.

11. Quantitation of Anchorage-Independent Growth (see section 13 if cell recovery/re-plating is desired)

11.1 Add 125 µL (for a 96-well plate) or 625 µL (for a 24-well plate) of Lysis Buffer to each well.

11.2 Pipette the entire volume of the well 15-20 times to mix thoroughly, solubilizing the agar matrix and lysing the cells completely.

11.3 Transfer 50 µL of the mixture to a 96-well plate suitable for fluorescence measurement.

Δ Note: This same solution may be assayed for alkaline phosphatase activity (see step 12.3 of section 12 below).

11.4 Prepare sufficient PBS/GR dye solution for all samples by diluting the dye 1:200 in PBS (for example, add 5 µL dye to 995 µL of PBS).

11.5 Add 100 µL of PBS/GR dye solution to each well (already containing 50 µL of solution). Incubate the plate at room temperature for 30 minutes.

11.6 Pipette each well 7-10 times to ensure a homogeneous mixture.

11.7 Read the plate in a 96-well fluorometer using a 485/520 nm filter set.

12. Quantitation of Alkaline Phosphatase Activity (see section 13 if cell recovery/re-plating is desired)

12.1 Add 125 µL (for a 96-well plate) or 625 µL (for a 24-well plate) of Lysis Buffer to each well.

12.2 Pipette the entire volume of the well 15-20 times to mix thoroughly, solubilizing the agar matrix and lysing the cells completely.

12.3 Transfer 50 µL of the mixture to a 96-well microtiter plate.

Δ Note: This same solution may be assayed for cell quantitation (see step 11.3 of section 11 above).

12.4 Add 50 µL of AP Activity Assay Substrate to each well (already containing 50 µL of solution). Incubate the plate at 37°C for 30 minutes.

12.5 To stop the reaction, add 50 µL of AP Stop Solution to each well.

12.6 Pipette each well 7-10 times to ensure a homogeneous mixture.

12.7 Measure the absorbance at 405 nm in a 96-well microtiter plate reader.

13. Cell Recovery and Re-plating

13.1 Add 125 µL (for a 96-well plate) or 625 µL (for a 24-well plate) of 1X Matrix Solubilization Solution to each well.
13.2 Pipette each well 10-12 times to mix thoroughly.
13.3 Transfer the entire mixture to at least 20 volumes of standard culture medium (for example, 1 mL would be transferred to 20 mL media).
13.4 Pipette the mixture vigorously 7-10 times.
13.5 Centrifuge the cell pellet and aspirate the media supernatant.
13.6 Resuspend the cell pellet in another 20 volumes of standard culture medium.
13.7 Repeat steps 13.4-13.6.
13.8 Resuspend the pellet and transfer to a tissue culture flask or dish.
13.9 Transfer to a cell culture incubator.

14. Calculation of Anchorage-Independent Growth

14.1 Compare RFU values with the Cell Dose Curve and extrapolate the cell concentration.

14.2 Calculate the Total Cell Number/Well

Total Cells/Well = cells/mL x 0.050 mL/well

For example: If you extrapolate your RFU value from your cell dose curve and determine you have 500,000 cells/mL in your sample.

Total Cells/Well = 500,000 cells/mL x 0.050 mL/well = 25,000 cells/well

15. Typical Data

Typical data provided **for demonstration purposes only**.

The following figures demonstrate typical results with the 96-well Stem Cell Colony Formation Assay. Fluorescence measurement was performed on SpectraMax Gemini XS Fluorometer (Molecular Device) with a 485/520 nm filter set and 530 nm cutoff.

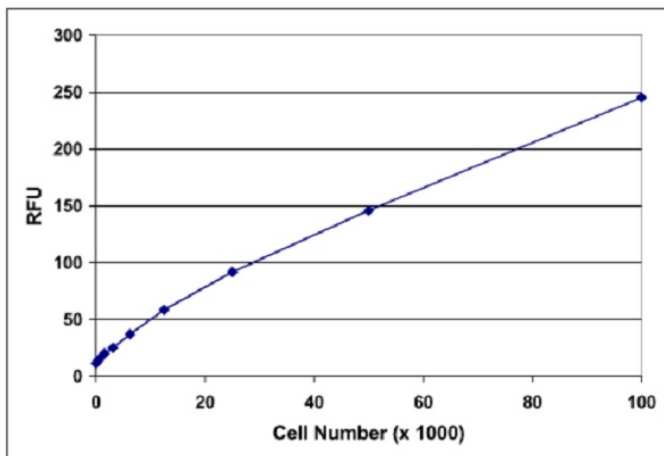


Figure 1. ES-D3 Cell Dose Curve. Murine embryonic stem cells (ES-D3) were resuspended at 12 x 10^6 cells/mL and titrated 1:2 in culture medium, followed by addition of Cell Dose Curve Solution, Matrix Solubilization Solution, Lysis Buffer, and GR Dye detection (as described in the Cell Dose Section). Results are shown by actual cell number in CyQuant Detection.

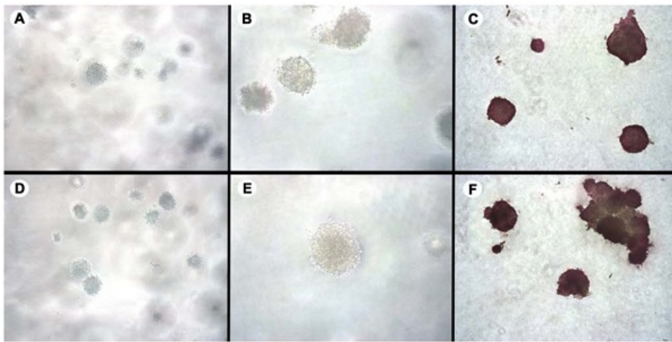


Figure 2. ES-D3 Colony Formation. Murine embryonic stem cells (ES-D3) were cultured for 7 days in the presence (D-F) or absence (A-C) of LIF, according to the assay protocol. Phase contrast images demonstrate colonies at 10X magnification (A, D), 20X magnification (B, E), and 20X magnification with AP staining (C, F).

17. Notes

Technical Support

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