ab325583 – *In Vitro* Tumor Sensitivity Assay (Soft Agar Colony Formation)

A stringent, anchorage-independent model for chemosensitivity testing and potential anti-cancer drug screening.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab325583

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components.

Materials Supplied

Item	Quantity 96 Tests	Storage Condition
10X Agar Matrix Solution	10 mL	+4°C
Matrix Diluent	4 mL	+4°C
5X DMEM Medium	5 mL	+4°C
Detergent Solution	10 mL	+4°C
MTT Solution	1 mL	+4°C
1X Matrix Solubilization Buffer	20 mL	+4°C

Materials Required, Not Supplied

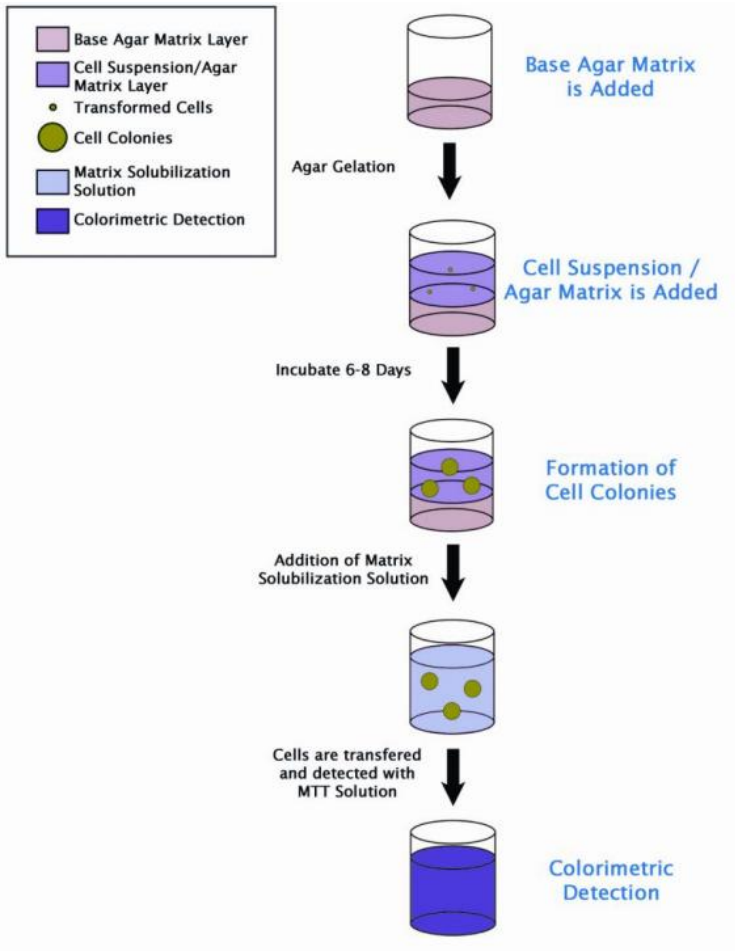
These materials are not included in the kit, but will be required to successfully utilize this assay:

- Tumor Cells (cancer cell line or cells prepared from solid tumor)
- Anti-cancer Agents (e.g., Taxol, 5-Fluorouracil, anticancer mAb or siRNA)
- 37°C Incubator, 5% CO_2 Atmosphere
- Light Microscope
- 96-well Microtiter Plate Reader
- 37°C and boiling water baths

Preparation of Reagents

- 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. Sterile filter the 2X media to 0.2 µm.
  ∆***Note:*** *You may substitute your own medium in place of the DMEM we provide, but ensure that it is at a 2X concentration.*
- 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.

Assay Principal



Assay Protocol (must be under sterile conditions)

Preparation of Base Agar Matrix Layer

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.
2. Warm the 2X DMEM/20% FBS medium (see Preparation of Reagents section) to 37°C in a water bath. Allow at least 30 minutes for the temperature to equilibrate.
3. According to Table 1 (below), prepare the desired volume of Base Agar Matrix Layer in the following sequence:

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

∆***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**

Table 1. Preparation of Base Agar Matrix Layer.

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

∆***Notes:***
- *Work quickly with the layer to avoid gelation. Also, try to avoid adding air bubbles to the well.*
- *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.*

6. Transfer the plate to 4°C for 30 minutes to allow the Base Agar Matrix Layer to solidify.
7. Prior to adding the Cell Suspension/Agar Matrix Layer (next section), allow the plate to warm to room temperature for 30 minutes.

Addition of Cell Suspension/Agar Matrix Layer (under sterile conditions)

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.
2. Warm the 2X DMEM/20% FBS medium (see Preparation of Reagents section) and Matrix Diluent to 37°C in a water bath. Allow at least 30 minutes for the temperature to equilibrate.
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.
4. According to Table 2 (below), prepare the desired volume of Cell Suspension/Agar Matrix Layer in the following sequence:
   a. In a sterile tube, add the appropriate volume of 2X DMEM/20% FBS medium.
   b. Next, add the corresponding volume of Matrix Diluent. Mix well.
   c. Next, add the corresponding volume of 10X Agar Matrix Solution. Mix well.
   d. Finally, add the corresponding volume of cell suspension. Mix well.

∆***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	0.375	0.25	3.75	**50**
0.875	0.688	0.188	0.125	1.875	**25**

Table 2. Preparation of Cell Suspension/Agar Matrix Layer.

5. After mixing, incubate the Cell Suspension/Agar Matrix Layer at room temperature for 5 minutes.
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 (previous section).

∆***Notes:***
- *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.*
- *Always include negative control wells that contain no cells in the Cell Suspension/Agar Matrix Layer.*

7. Transfer the plate to 4°C for 20 minutes to allow the Cell Suspension/Agar Matrix Layer to solidify.
8. Allow the plate to warm to room temperature for 30 minutes.
9. Add 50 µL of culture medium containing anti-cancer agents (e.g. Taxol, 5-Fluorouracil, mAb, etc.) to each well.
10. Incubate the cells for 6-8 days at 37°C and 5% CO_2. Examine the colony formation under a light microscope.

Quantitation of Anchorage-Independent Growth

1. Add 125 µL of 1X Matrix Solubilization Solution to each well.
2. Pipette the entire volume of the well 10-12 times to mix thoroughly and solubilize the agar matrix completely.
3. Transfer 100 µL of the mixture to a 96-well microtiter plate.
4. Add 10 µL of MTT Solution to each well. Pipette each well 7-10 times to ensure a homogeneous mixture.
5. Incubate the plate for 2-4 hours at 37°C and 5% CO_2.
   ∆***Note:*** *Under the microscope, a purple precipitate should be visible within the cells.*
6. Add 100 µL of Detergent Solution to each well.
7. Incubate the plate in the dark for 2-4 hours at room temperature, with gentle shaking.
8. Pipette each well 7-10 times to ensure a homogeneous mixture.
9. Measure the absorbance at 570 nm in a 96-well microtiter plate reader.

Cell Dose Curve (optional)

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.
2. Warm the 2X DMEM/20% FBS medium (see Preparation of Reagents section) and Matrix Diluent to 37°C in a water bath. Allow at least 30 minutes for the temperature to equilibrate.
3. Harvest and resuspend cells in culture medium at 5 - 10 x 10^6 cells/mL.
4. Prepare a serial 2-fold dilution in culture medium, including a blank without cells.
5. Transfer 50 µL of each dilution to a 96-well plate.
6. According to Table 3 (below), prepare the desired volume of Cell Dose Curve Solution in the following sequence:
   a. In a sterile tube, add the appropriate volume of 2X DMEM/20% FBS medium.
   b. Next, add the corresponding volume of sterile water. Mix well.
   c. Next, add the corresponding volume of Matrix Diluent. Mix well.
   d. Finally, add the corresponding volume of 10X Agar Matrix Solution. Mix well.

∆***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 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**

Table 3. Preparation of Cell Dose Curve Solution.

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 5).
8. Add 125 µL of 1X Matrix Solubilization Solution to each well. Pipette each well 10-12 times to mix thoroughly.
9. Transfer 100 µL of the mixture to a 96-well microtiter plate.
10. Add 10 µL of MTT Solution to each well. Pipette each well 7-10 times to ensure a homogeneous mixture.
11. Incubate the plate for 2-4 hours at 37°C and 5% CO_2.
    ∆***Note:*** *Under the microscope, a purple precipitate should be visible within the cells.*
12. Add 100 µL of Detergent Solution to each well.
13. Incubate the plate in the dark for 2-4 hours at room temperature, with gentle shaking.
14. Pipette each well 7-10 times to ensure a homogeneous mixture.
15. Measure the absorbance at 570 nm in a 96-well microtiter plate reader.

Technical Support

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