

## ab325582 – Cell Transformation Assay (Cell Recovery Compatible)

A robust system for measuring *in vitro* drug sensitivity, screening oncogenes and cell transformation inhibitors, while allowing for transformed cell recovery.  
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: [www.abcam.com/ab325582](http://www.abcam.com/ab325582)

**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
10X Matrix Solubilization Solution	1.8 mL	+4°C
Detergent Solution	10 mL	+4°C
MTT Solution	1 mL	+4°C

### Materials Required, Not Supplied

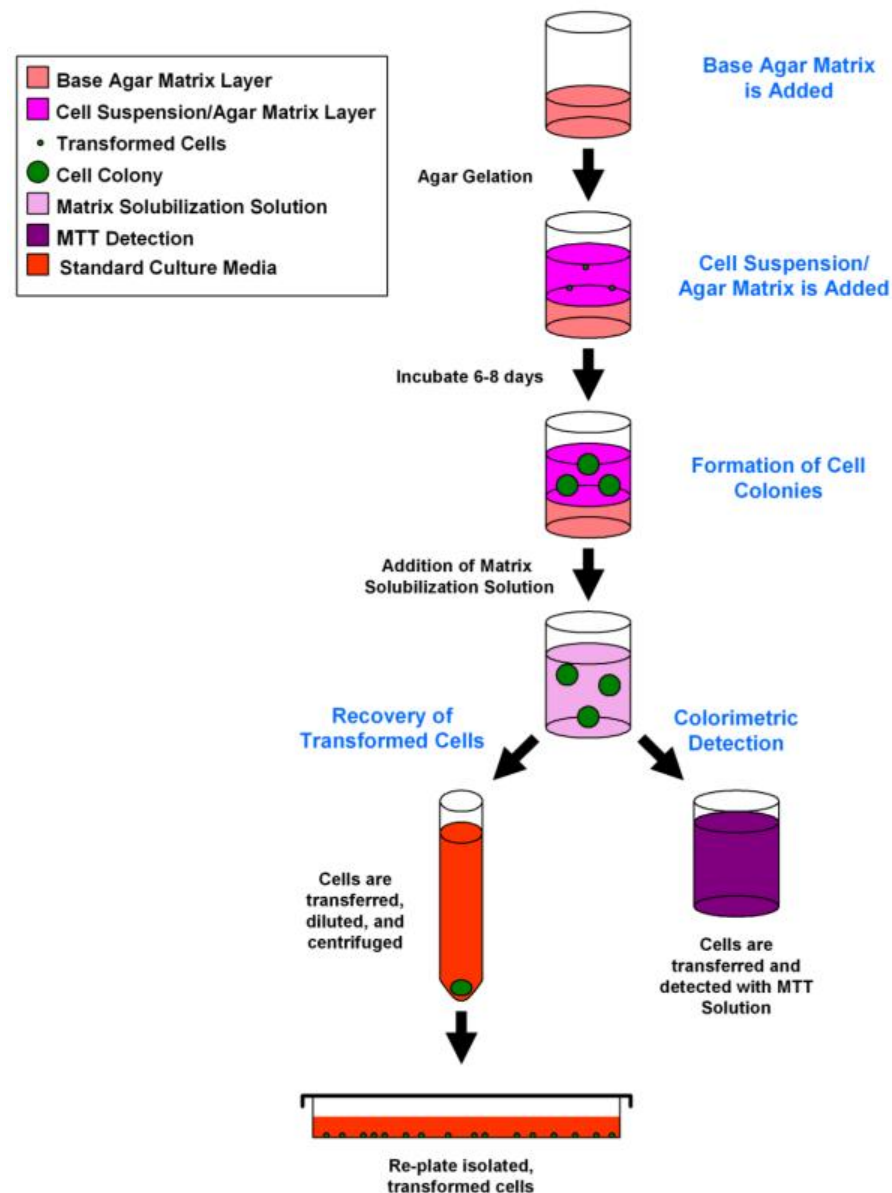
These materials are not included in the kit, but will be required to successfully utilize this assay:

- Cells and Culture Medium
- 37°C Incubator, 5% CO<sub>2</sub> Atmosphere
- Light Microscope
- 96-well Microtiter Plate Reader
- 37°C and boiling water baths
- (Optional) Positive Control cells such as NIH 3T3 (Ras G12V)

### 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.  
**ΔNote:** You may substitute your own medium in place of the DMEM we provide, but ensure that it is at a 2X concentration.
- 1X Matrix Solubilization Solution: Prepare a 1X Matrix Solubilization Solution by diluting the provided 10X stock 1:10 in sterile cell culture grade water. Sterile filter the 1X solution to 0.2 µm.
- 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)

The assay protocol below is written for a 96-well format. Refer to Table 1 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
1X Matrix Solubilization Solution	125	250	625	1250	2500

**Table 1.** Dispensing Volumes of Different Plate Formats.

### Preparation of Base Agar Matrix Layer

- 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.
- 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.
- According to Table 2 (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.

**ΔNote:** 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 2.** Preparation of Base Agar Matrix Layer.

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

#### ΔNotes:

- 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.

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

- 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.
- 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.
- Harvest and resuspend cells in culture medium at 0.1 - 1 x 10<sup>6</sup> cells/mL. Keep the cell suspension warm in a 37°C water bath.
- According to Table 3 (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.

**ΔNote:** 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 3.** Preparation of Cell Suspension/Agar Matrix Layer.

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

#### ΔNotes:

- 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.
- Transfer the plate to 4°C for 20 minutes to allow the Cell Suspension/Agar Matrix Layer to solidify.
  - Allow the plate to warm to room temperature for 30 minutes.

9. Add 50  $\mu$ L of culture medium containing cell growth activator(s) or inhibitor(s) to each well.
10. Incubate the cells for 6-8 days at 37°C and 5% CO<sub>2</sub>. Examine the colony formation under a light microscope.

### Quantitation of Anchorage-Independent Growth (skip to the next section if cell recovery/re-plating is desired)

1. Add 125  $\mu$ L 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  $\mu$ L of the mixture to a 96-well microtiter plate.
4. Add 10  $\mu$ L of MTT Solution to each well. Pipette each well 7-10 times to ensure a homogeneous mixture. Incubate the plate for 2-4 hours at 37°C and 5% CO<sub>2</sub>.  
 **$\Delta$ Note:** Under the microscope, a purple precipitate should be visible within the cells.
5. Add 100  $\mu$ L of Detergent Solution to each well.
6. Incubate the plate in the dark for 2-4 hours at room temperature, with gentle shaking.
7. Pipette each well 7-10 times to ensure a homogeneous mixture.
8. Measure the absorbance at 570 nm in a 96-well microtiter plate reader.  
 **$\Delta$ Note:** If values are low, continue the room temperature incubation for up to 24 hours.

### Cell Recovery and Re-plating (under sterile conditions)

1. Add 125  $\mu$ L of 1X Matrix Solubilization Solution to each well.
2. Pipette each well 10-12 times to mix thoroughly.
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).
4. Pipette the mixture vigorously 7-10 times.
5. Centrifuge the cell pellet and aspirate the media supernatant.
6. Resuspend the cell pellet in another 20 volumes of standard culture medium.
7. Repeat steps 4-6.
8. Resuspend the pellet and transfer to a tissue culture flask or dish.
9. Transfer to a cell culture incubator.

### 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<sup>6</sup> cells/mL.
4. Prepare a serial 2-fold dilution in culture medium, including a blank without cells.
5. Transfer 50  $\mu$ L of each dilution to a 96-well plate.
6. According to Table 4 (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.

**$\Delta$ 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 4.** Preparation of Cell Dose Curve Solution.

7. Immediately dispense 125  $\mu$ L 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  $\mu$ L of 1X Matrix Solubilization Solution to each well. Pipette each well 10-12 times to mix thoroughly.
9. Transfer 100  $\mu$ L of the mixture to a 96-well microtiter plate.
10. Add 10  $\mu$ L of MTT Solution to each well. Pipette each well 7-10 times to ensure a homogeneous mixture. Incubate the plate for 2-4 hours at 37°C and 5% CO<sub>2</sub>.  
 **$\Delta$ Note:** Under the microscope, a purple precipitate should be visible within the cells.
11. Add 100  $\mu$ L of Detergent Solution to each well.
12. Incubate the plate in the dark for 2-4 hours at room temperature, with gentle shaking.
13. Pipette each well 7-10 times to ensure a homogeneous mixture.
14. Measure the absorbance at 570 nm in a 96-well microtiter plate reader.  
 **$\Delta$ Note:** If values are low, continue the room temperature incubation for up to 24 hours.

### Technical Support

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