INTRODUCTION

AIM idenTx 3 chips and idenTx 9 plates allow the immune cells migrate to and interact with the cancer cells within a 3D hydrogel. This is particularly useful for the studies in immunotherapy. The idenTx 9 encompasses three individual idenTx 3 chips and a idenTx Holder, enabling 9 independent experiments on a single standard SBS-format plate. This protocol covers the calculations and techniques for seeding cancer cells in a 3D hydrogel followed by introducing immune cells in the adjacent media channel. The co-culture of human hepatocellular carcinoma (HepG2) and T cells is used here as an illustrative example.

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PREPARING & FILLING COLLAGEN GEL WITH CANCER CELLS ⏱ TIMING 50 min

MATERIALS

Reagents

- 10 X PBS with phenol red (see REAGENT SETUP in the General Protocol; Life Technologies, Cat. No. 70011044)
- Sodium hydroxide solution, 0.5 M (see REAGENT SETUP in the General Protocol; Sigma-Aldrich, Cat. No. 221465)
- Sterile deionized water (Thermo Water Purifying System)
- Collagen type I, rat tail (Corning Life Science, Cat. No. 354236)
- R10 cell culture medium (see REAGENT SETUP at the end of this protocol)
- 1X PBS (Life Technologies, Cat. No. 70011044)
- Trypsin (Life Technologies, Cat. No. 25300054)

Others

- Cancer cells e.g. HepG2 (GFP-labelled, **Option 1**: dispersed; **Option 2**: aggregated)
- 1.5 ml microcentrifuge tube
- Pocket size pH meter (Hach, Cat. No. H138) or pH papers (Sigma Aldrich, Cat. No. 37144)
- Ice bucket or styrofoam box
- Ice
- idenTx 3 or idenTx 9
- idenTx holders or humidified chambers

Calculations for the Preparation of Collagen with Cells

i. The following steps are calculated based on a collagen stock solution of 3.75 mg/ml, but any other concentration in the range of 3 - 4.5 mg/ml may still be used.

ii. Determine the concentration and the total volume of the collagen solution needed for your application, e.g. 2.5 mg/ml and 200 µl respectively

Reminder *The concentration, pH value and total volume of the final collagen solution can be adjusted for different applications.*

iii. The volume of cancer cell suspension to be added into the collagen gel solution is typically in the range of 10 – 20 µl per 200 µl of collagen gel solution. For ease of calculation, you can fix the volume of cell suspension as E = 20 µl.

Reminder *The volume of cell suspension can be adjusted for different applications but cannot exceed the volume of water needed to dilute the collagen stock solution to the final concentration. Avoid setting the volume of cell suspension, E too low as it is hard to work with low volume of cell suspension, especially if it is concentrated.*

iv. Calculate the volume of 10X PBS needed to obtain 200 µl of 1X collagen solution based on the equation below, A = 18 µl.

$$\frac{10A + E}{Total\ Volume} = 1$$

$$A = \frac{Total\ Volume - E}{10} = \frac{200 - 20}{10} = 18\mu l$$

v. The volume of collagen, B is calculated based on the equation below, B = 133.3 µl

$$3.75\frac{mg}{ml} \times B = \frac{2.5mg}{ml} \times 200\mu l$$

vi. The volume of 0.5 M NaOH needed to adjust the pH of the solution is a variable that usually falls between 4 to 8 µl and the exact number can be determined through iterative method. For a collagen stock solution of 3.75 mg/ml, the volume of 0.5 M NaOH solution needed, C = 8 µl

vii. Add sterile deionized water to dilute the collagen solution to the desired concentration without affecting the pH value. The volume of deionized water, D is calculated last by using the equation below.

$$D = Total\ Volume - A - B - C - E$$

$$D = 200\mu l - 18\mu l - 133.3\mu l - 8\mu l - 20\mu l = 20.7\mu l$$

Iterative method to determine the volume of 0.5 M NaOH solution

1. Perform the following steps in a laminar flow hood and every item should be sterilized beforehand.

2. Keep 10X PBS with phenol red, medium, 0.5 M NaOH solution, deionized water and collagen stock solution on ice throughout the process.

Reminder *Make sure the color of 10X PBS with phenol red is homogeneous before use as the color of 10X PBS with phenol red changes when it is about to freeze.*

3. Add 18 µl of 10 X PBS with phenol red into a microcentrifuge tube.

4. Add 20 µl of culture medium into the microcentrifuge tube

5. Add the calculated volume of collagen, B into the microcentrifuge tube.

6. Add an estimated amount of 0.5 M NaOH solution into the microcentrifuge tube. The recommended starting volume is 4 µl to make 200 µl of 2.5 mg/ml collagen solution at pH 7.4.

7. Mix thoroughly with a micropipette.

! Critical *Pipette up and down until the color is homogeneous throughout (if unsure, pipette at least 100 times).*

! Critical *Pipette up and down with care to avoid the generation of bubbles.*

! Critical *Keep the microcentrifuge tube on ice while mixing to prevent any unwanted polymerization of the collagen gel.*

8. Check the color of the mixed solution. Phenol red is a pH indicator itself and the desired color for pH7.4 is faint pink.

9. Discard the mixed solution if the mixed solution is purple or red, which indicates a high pH value. Start again with a lower volume of 0.5 M NaOH solution.

10. Add 0.5 M NaOH solution into the mixed solution in a small stepwise manner (each addition is not more than 0.5 µl) if the mixed solution is yellow, which indicates a low pH value. Mix thoroughly with each addition of 0.5 M NaOH solution. Repeat this until the color changes to faint pink.

! Critical *Make sure no residue is left in the pipette tip as the pH value is highly sensitive to the addition of 0.5 M NaOH solution.*

11. If a pocket size pH meter is available, add a drop of mixed solution (~10 µl depends on the model of pH meter) to measure the pH value. Otherwise, add 10 µl to 20 µl of mixed solution on a pH paper and match it to the color chart.

Table 1 An example of collagen gel recipe

12. Confirm the pH value is within ±0.1 of the target value with a pH meter. Otherwise, make sure the color of mixed solution is faint pink and the pH paper readout lies between pH7 and pH8.

13. Calculate the volume of deionized water required and add it into the mixed solution.

14. The collagen gel recipe may be used for future experiments as long as the same reagents are used. For any change of reagent, a new recipe has to be developed again.

Reagents	Volume
10X PBS	18 µl
Collagen	133.3 µl
0.5 M NaOH	8 µl
Deionized water	20.7 µl
Cell suspension	20 µl

Option 1: Preparing collagen gel (with dispersed cells) ⏱ **TIMING 10 min**

15. The target seeding concentration in the gel for HepG2 is 2.5 M cells /ml, for example. Please adjust the concentration accordingly for your cancer cells of interest.

16. Do a backward calculation to determine the concentration of cell suspension, c_{cell} = 25 M cells/ml

$$c_{cell} \times E = Target\ Seeding\ Concentration \times Total\ Volume$$

$$c_{cell} \times 20\mu l = \frac{2.5 M cells}{ml} \times 200\mu l$$

$$c_{cell} = 25 M cells/ml$$

17. Trypsinize cells as per protocol and resuspend the cells in culture medium to obtain 25 M cells/ml.

18. Keep 10X PBS with phenol red, collagen stock solution, 0.5 M NaOH solution, deionized water and cell suspension on ice and add them into a microcentrifuge tube (on ice) sequentially according to the pre-determined collagen gel recipe.

19. Mix the solution thoroughly by using a micropipette to get a collagen solution with homogeneous faint pink color.

Option 2: Preparing collagen gel (with cell aggregates) ⏱ **TIMING 15 min**

20. The target seeding concentration in the gel for HepG2 aggregates is 30 - 40 aggregates per site, for example. Please adjust the concentration accordingly for your cancer aggregates of interest.

21. Do a backward calculation to determine the concentration of cell suspension, c_{cell} = 40 aggregates per injection ~ 40 aggregates per 10 µl

$$c_{cell} \times E = Target\ Seeding\ Concentration \times Total\ Volume$$

$$c_{cell} \times 20\mu l = \frac{40\ aggregates}{10\ \mu l} \times 200\mu l$$

$$c_{cell} = 40\ aggregates/\mu l$$

22. Collect the cell aggregates from aggregate-forming platforms (e.g. low attachment plates) and filter the cell aggregates with 40 µm and 100 µm filters to get the right-size aggregates (between 40 – 100 µm). Resuspend the cells in culture medium to obtain 40 aggregates/µl.

23. Keep 10X PBS with phenol red, collagen stock solution, 0.5 M NaOH solution, deionized water and cell suspension on ice and add them into a microcentrifuge tube (on ice) sequentially according to the pre-determined collagen gel recipe.

24. Mix the solution thoroughly by using a micropipette to get a collagen solution with homogeneous faint pink color.

Filling collagen gel (with cells) ⏱ **TIMING 40 min**

25. Assemble idenTx 3 into an idenTx holder (see Instructions For Use for the idenTx holder included in the package). The idenTx 9 is ready-to-use.

26. Draw 10 µL of collagen solution (with cells) with a 1- 10 µL micropipette. Make sure the collagen solution is kept on ice at all times.

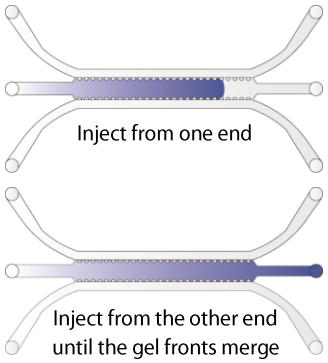
! **Critical** *Limit the volume of collagen solution to 10 µl to prevent the collagen solution from overflowing into media channels.*

27. Fill hydrogel from either gel inlet:

! **Critical** *Hold the plunger firmly while removing the micropipette from the inlets, otherwise the negative pressure will suck the collagen up.*

! **Critical** *Inject the collagen solution smoothly to ensure the cells distribute evenly in the gel.*

a. **Option 1**: Fill collagen solution from either one of the inlets and stop near the end of posts. Fill from the other inlet until the gel fronts merge. This method is recommended for new users.



b. **Option 2**: Fill collagen solution from one side all the way to the other side. Continue to push the collagen solution in gently until it reaches the other inlet. This method ensures that the gel is being filled homogeneously but it requires greater control over pipetting pressure (especially when the collagen solution reaches the opposite inlet) to prevent the collagen solution from overflowing into the flanking media channels.

28. Add 6 ml of water into the reservoirs of the idenTx holder. Alternatively, prepare a humidified chamber to house the chips (e.g. by adding water into a pipette tip box until approximately 1/3 is filled; both water and pipette tip box should be sterile).

29. If humidified chambers are used, pre-warm the humidified chambers to 37 °C by putting them in an incubator.

30. Place the gel-filled chips (on idenTx holders or in humidified chambers) into a 37 °C incubator and incubate for half an hour to allow the polymerization of collagen to take place.

! Critical *idenTx 3 or idenTx 9 is laminated with a gas-permeable film that enables gas exchange to take place. The bottom of the chips should therefore be exposed to allow for air circulation.*

Reminder *It is not necessary to pre-warm idenTx holders as the idenTx holders allow direct contact of idenTx 3 or idenTx 9 with warm air when placed in an incubator.*

Reminder *The polymerization time can be optimized to suit your specific application.*

! Critical *Temperature will affect collagen polymerization and 37 °C is recommended for most applications.*

! Critical *Chips with unpolymerized gel must be handled with care. Excessive agitation or impact may cause the unpolymerized gel to leak out of the gel channel.*

HYDRATING MEDIA CHANNELS ⏱ TIMING 5 min

MATERIALS

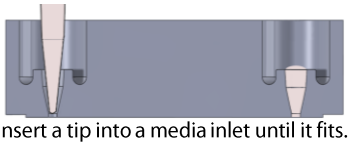
Reagents

- R10 cell culture medium (see REAGENT SETUP at the end of this protocol)

Others

- Collagen-filled idenTx 3 or idenTx 9

31. After incubation, insert a pipette tip into either inlet of the media channel and push gently until the tip fits. Inject 15 µl of medium into the channel. Due to surface tension, the injected medium will form a spherical cap at the opposite inlet. Repeat this step for the other channel.



Insert a tip into a media inlet until it fits. Inject medium till it reaches the opposite

! **Critical** *Hold the plunger firmly while removing the micropipette from the inlets, otherwise the negative pressure will suck the medium up.*

! **Critical** *Do not inject more than 20 µl of medium at this step or the high injection pressure may disrupt the collagen gel.*

32. Top up 60 µl of medium into each port.

33. Keep the chips in a 37 °C incubator for 24 h.

SETTING A BASELINE FOR LIVE-DEAD COUNT ⏱ TIMING 130 min

MATERIALS

Reagents

- R10 Cell culture medium with DRAQ7 (Biolegend, Cat. No. 424001)

34. Replace the medium in the chips 2 h before imaging the cells. Use cell culture medium that is supplemented with 3 µM DRAQ7 (final concentration) to label dead HepG2 cells.

35. Remove old medium from all 4 ports by carefully aspirating the old medium out from the troughs. To replace the old medium in a media channel, add 70 µl of DARQ7-supplemented medium into one port and then add 50 µl into the opposite connected port. Repeat this for the other channel.

Always remove medium from troughs

! **Critical** *The differential volumes in the two ports allow the replacement of medium to take place in the channel. The minimum volume of medium is 30 µl to ensure the inlets are covered and the troughs are wetted. If less than 30 µl of medium is used, the surface tension at the inlets will prevent the medium from flowing through the channel. We recommend using 50 µl of medium for easier handling.*

! **Critical** *Do NOT aspirate medium from inlets to avoid accidental removal of medium from the channels.*

36. Keep the chips in an incubator for 2 h.

37. Image the cancer cells by using confocal microscopy or high content imaging system to obtain the initial live-dead count of cancer cells in prior to the introduction of T cells.

Reminder *Cancer cells that are pre-labelled with GFP (or any other non-far red fluorescent label) yield more precise live-dead count*

SEEDING T CELLS ⏱ TIMING 90 min

MATERIALS

Reagents

- AIM-V cell culture medium (see REAGENT SETUP at the end of this protocol)
- RPMI 1640 medium (Thermo Fisher Scientific, Cat. No. 11835030)
- CellTracker Violet BMQC Dye (ThermoFisher Scientific, Cat. No. C10094)
- DRAQ7 (Biolegend, Cat. No. 424001)

Others

- Collagen-filled idenTx 3 or idenTx 9
- T Cells

38. Pool T cells as per protocol. Briefly, collect T cells from the culture flasks or multi-well plates and then count the number of live cells using trypan blue. Pellet the cells by centrifuging at 1400 rpm for 5 min at RT.

39. Use 0.5 ml of RPMI 1640 (with 3 µM of CellTracker Violet BMQC dye) to resuspend every 1.5 M of T cells in the microcentrifuge/falcon tubes.

40. Incubate T cells in an incubator for 30 min with gentle shaking every 15 min.

41. Top up the cell suspension with equal-volume of AIM-V medium and then pellet the cells by centrifuging at 1400 rpm for 5 min at RT

42. Wash the T cells by using 0.5 ml of AIM-V medium to resuspend every 1.5 M of T cells in the microcentrifuge/falcon tubes. Incubate T cells in an incubator for another 30 min.

43. Top up the cell suspension with equal-volume of AIM-V medium and count the number of live cells using trypan blue. Pellet the cells by centrifuging at 1400 rpm for 5 min at RT

44. Resuspend the cells in AIM-V medium at 6 M cells/ml

45. Change the old medium in the chips to AIM-V medium with 3 µM DRAQ7 (final concentration). Remove old medium from all 4 ports by carefully aspirating the old medium out from the troughs. To replace the old medium in a media channel, add 70 µl of DARQ7-supplemented AIM-V medium into one port and then add 50 µl into the opposite connected port. Repeat this for the other channel.

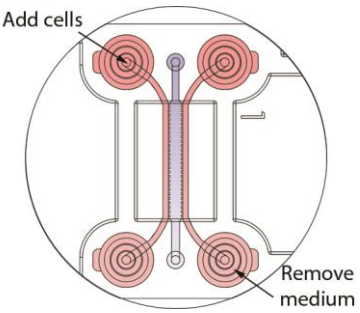
46. Use a micropipette to withdraw 30 µl of T cell suspension. Position the tip near the inlet of media channels and inject the cell suspension.

Reminder *Ports must be filled with medium before seeding cells into the media channels. Filled ports allow the cell suspension to flow into the media channels.*

Position the pipette tip at media inlets while injecting cell suspension

! Critical *Do not insert the tip completely into the inlets to avoid introducing cells into the media channels at a high flow rate. High flows will not allow cells to settle along the channel, resulting in uneven distribution.*

47. Withdraw 20 µl of medium from the port that is **diagonally opposite** to the one that is injected with T cells. The 50 µl of volume difference (30 µl of cell suspension added and 20 µl of medium withdrawn) creates a height difference between the two media channels thus generating interstitial flow across the gel that helps the attachment of T cells on the gel interface.



Add T cell suspension into one of the media inlets and then withdraw culture medium from the diagonally opposite port

! Critical *Lay chips (on idenTx holders or in humidified chambers) on a flat surface while seeding cells into idenTx 3 or idenTx 9. Inclination of the chips affects the flow in the media channel, thus disturbing cell distribution.*

48. Visual inspection under a microscope is recommended. If the cell distribution is not optimal for your application, adjust the concentration of the cell suspension and repeat the seeding steps.

? Troubleshooting (see Table 2 for troubleshooting advice)

49. Keep the chips in an incubator for 15 h before the end-point imaging.

Reminder *Live (time-lapse) imaging can be done by using microscopes with on-stage incubators*

QUANTIFICATION OF T CELL KILLING EFFICIENCY ⏱ TIMING Variable

In order to quantify the T cell killing efficiency in idenTx 3 or idenTx 9, we recommend labelling the cells with appropriate fluorophores (such as GFP-labelling for cancer cells and CellTracker Violet BMQC Dye for T cells) to visualize them. Bright field, phase contrast and epifluorescence microscopy are all compatible with idenTx 3 or idenTx 9 but three-dimensional imaging techniques such as confocal microscopy is preferred due to the nature of this assay. The following quantification method uses images taken from confocal microscopy as an illustrative example.

LIVE & DEAD COUNT OF DISPERSED CANCER CELLS

The live and dead count of cancer cells in the chips is a direct indication for the T cell killing efficiency. The live and dead counts before the addition of T cells and after the 15 h incubation with T cells are compared. The greater the difference in the live and dead counts between the two time points, the more potent the T cells.

50. We recommend using confocal images of cancer and T cells that are fluorescently tagged to quantify the live and dead counts.

Reminder *DRAQ7 that stains the nuclei of dead cells is used here to identify dead cells.*

51. Use CellProfiler (http://cellprofiler.org) to estimate the average diameter of dispersed cancer cells in the gel by projecting a stack of confocal images into a single maximum-intensity-projection (MIP) image. The mean diameter of cancer cells provides a more accurate estimate on the total number of cancer cells in the gel.

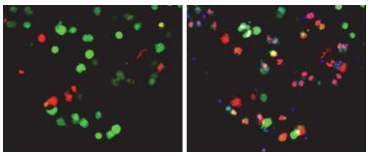


Figure 1 Confocal images of cancer cells at 0h (left) and the same region 15 h after addition of T cells (right). Cancer cells are labelled with GFP (green), dead cells are labelled with DRAQ7 (red) and T cells are labelled with CellTracker Violet BMQC (blue). Adapted from Pavesi et al. 2017[1]

52. Use the built-in spot detection function in Imaris (http://bitplane.com) to capture the total number of cells and their corresponding fluorescence intensities.

53. Plot the mean fluorescence intensity (MFI) of DRAQ7 against the MFI of GPF to get the live and dead cell distribution. The group with 10% DMSO (causes cancer cell death) can be used as a positive control to determine the threshold of the MFI that differentiates live and dead cells.

    Alternative option: Use 3D Objects Counter plugin (http://imagej.net/3D_Objects_Counter) in ImageJ to obtain the integrated density. Adjust the threshold value and size filter to make sure the cells in the region of interest are captured. Plot the integrated density of DRAQ7 against the integrated density of GFP to get the live and dead cell distribution.

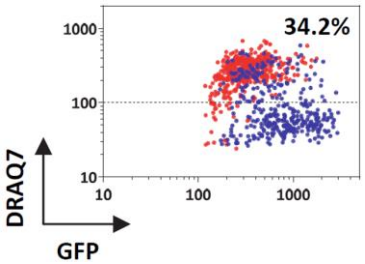


Figure 2 A scatterplot for the T cell group (blue dots) and the 10% DMSO control group (red dots). The MFI of DRAQ7 is plotted against the MFI of GFP. The cells with MFI of DRAQ7 > 100 are considered as dead cells in this experiment. Adapted from Pavesi et. al. 2017[1]

54. To quantify the killing efficiency of T cells, divide the number of live cancer cells at 15 h time point by the number of live cells at 0 h.

PERCENTAGE OF CELL DEATH OF CANCER AGGREGATES

Quantifying live and dead cells in cancer aggregates is usually less accurate as the cancer cells clump together. Therefore, the percentage of cell death is estimated based on the volumes of DRAQ7-positive cells and GFP-positive cells. The killing efficiency of T cells can be obtained by comparing the percentage of cell death before the addition of T cells with that after the 15 h incubation. The greater the difference in percentage of cell death between the two time points, the more potent the T cells.

55. We recommend using confocal images of cancer and T cells that are fluorescently tagged to quantify the cell volume.

56. Identify a particular cell aggregate under GFP channel, draw a region of interest (ROI) around the aggregate and create a corresponding reconstructed surface by using the Surface function in Imaris (http://bitplane.com). Therefore, the volume of the newly created surface can be determined.

57. Create a mask channel to confine the subsequent analysis for DRAQ7-positive cells within the same ROI. Repeat step 56 to create the corresponding reconstructed surface/s under DRAQ7 channel and determine the total volume of DARQ7-positive cells.

    Alternative option: Use ImageJ to identify the cell aggregate, define the ROI and duplicate the selected ROI into two new stacks: one with GFP-channel selected and the other with DRAQ7-channel selected. Use 3D Objects Counter plugin (http://imagej.net/3D_Objects_Counter) to analyze the two new stacks and determine the volumes of GFP-positive and DRAQ7-positive cells. Adjust the threshold value and size filter to make sure the cells in the region of interest are captured.

Reminder *DRAQ7 that stains the nuclei of dead cells is used here to identify dead cells.*

! Critical *The thresholding method/value greatly affects the value of volume. Make sure the image after thresholding is representative of your data.*

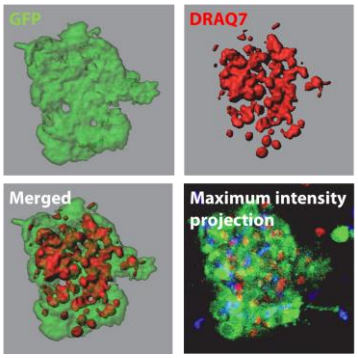


Figure 3 The reconstructed surfaces for GFP-positive cells (top left), DRAQ7-positive cells (top right) and the merged channel (bottom left). The maximum intensity projection (bottom right) is shown as a reference. Adapted from Pavesi et. al. 2017[1]

58. To determine the percentage of cell death, divide the volume of DRAQ7-positive cells by the volume of GFP-positive cells.

59. To determine the killing efficiency of T cells, compare the percentage of cell death before the addition of T cells with that after the 15 h incubation.

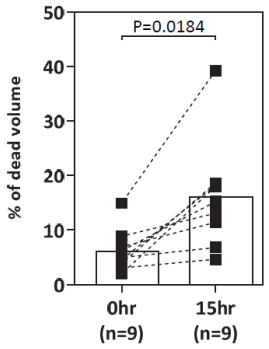


Figure 4 A representative bar graph shows that the percentage of dead volume is higher after the 15 h incubation with T cells. Adapted from Pavesi et. al. 2017[1]

TROUBLESHOOTING

Table 2 Troubleshooting advice

Step	Problem	Possible Reason	Solution
48.	Too many T cells in a channel	Concentration of cell suspension is too high	Start with cell suspension that is less concentrated
48.	Too few T cells in a channel	Concentration of cell suspension is too low	Increase the concentration of cell suspension or repeat the seeding steps (without modifying the concentration of cell suspension) until the target cell density is obtained
48.	T cells do not flow into the channel	Path of flow is blocked (e.g. by hydrogel that leaks into the media channel)	Inspect the chips under microscope before seeding T cells to identify the blocked part
		T cell suspension is not added into the media inlet so the majority of T cells settle at trough	Make sure cell suspension is injected into media inlets
48.	T cells do not adhere to the gel interface	The pressure head applied is insufficient	Increase the volume of cell suspension
48.	Debris clogs the gel interface	High number of impurities in the T cell suspension	Centrifuge the T cell suspension and resuspend them in filtered medium

REAGENT SETUP

R10 CELL CULTURE MEDIUM

Reagents

- RPMI 1640 medium (Thermo Fisher Scientific, Cat. No. 11835030)
- Heat-inactivated FBS (Thermo Fisher Scientific, Cat. No. 10082139)
- HEPES (Thermo Fisher Scientific, Cat. No. 15630106)
- Sodium pyruvate (Thermo Fisher Scientific, Cat. No. 11360070)
- Penicillin - Streptomycin (Thermo Fisher Scientific, Cat. No. 15140148)
- MeM amino acids without L-glutamine (Thermo Fisher Scientific, Cat. No. 11130051)
- MeM non-essential amino acids (Thermo Fisher Scientific, Cat. No. 11140050)
- Plasmocin (InvivoGen, Cat. No. ant-mpp)
- Puromycin (Clontech, Cat. No. 631305)

Others

- 0.2 µm 250 ml bottle top filter or 0.2 µm syringe filter

1. Supplement RPMI 1640 basal medium with 10% heat-inactivated FBS, 20 mM HEPES, 0.5 mM sodium pyruvate, 1% penicillin-streptomycin, 1X MeM amino acids, 1X MeM non-essential amino acids, 5 µg/ml plasmocin and 5 µg/ml of puromycin (to select GFP-transfected cells).

2. Sterilize R10 cell culture medium by passing through a 0.2 µm bottle top filter or syringe filter in a sterile laminar flow hood.

AIM-V CELL CULTURE MEDIUM

Reagents

- AIM-V medium (Thermo Fisher Scientific, Cat. No. 12055091)
- Human AB serum (Sigma-Aldrich, Cat. No. H4522)
- Recombinant IL-2 (R&D Systems, Cat. No. 202-IL-010)
- Acetic acid (Sigma-Aldrich, Cat. No. A6283)
- BSA (Sigma Aldrich, Cat. No. A9647)

Others

- 250 ml 0.2 µm bottle top filter or 0.2 µm syringe filter

3. Reconstitute rIL-2 at 100 µg/ml (2100000 IU/ml) in sterile 100 mM acetic acid with at least 0.1% of human or bovine serum albumin as carrier.

4. Supplement AIM-V medium with 2% human AB serum and 100 IU/ml rIL-2.

5. Sterilize the enriched AIM-V cell culture medium by passing through a 0.2 µm bottle top filter or syringe filter in a sterile laminar flow hood.

1. PAVESI, A., ET AL., *A 3D MICROFLUIDIC MODEL FOR PRECLINICAL EVALUATION OF TCR-ENGINEERED T CELLS AGAINST SOLID TUMORS.* JCI INSIGHT, 2017. **2**(12).