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Scriptwriter Name: Ruchira Mukherji

Project Page Link: https://review.jove.com/files_upload.php?src=21717268

Title: A Multimodal Framework to Assess Patient-Derived Colon Tumor Organoid–Immune Cell Co-Cultures

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NOTE: The article was not yet published at the time of the post-shoot, so the authors and affiliations may change later. Currently, it matches the list on the website

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes**

If **Yes**, can you record movies/images using your own microscope camera? **Yes**

NOTE: Videographer filmed the scope shots

Videographer's NOTE: In addition to scopes steps filmed on camera the authors provided high resolution still images of each scope shot.

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. Filming location: Will the filming need to take place in multiple locations? **Yes**

If **Yes**, how far apart are the locations? **Door to door time is a 5-minute walk**

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 19

Number of Shots: 57

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

Authors: The statements are edited for length and clarity. Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

INTRODUCTION:

Videographer's NOTE: Author provided narration of each step on audio channels 3&4.

REQUIRED: Summarize your research focus and the main questions it aims to answer.

- 1.1. **Chaoyuan Kuang:** This protocol establishes patient-derived colorectal organoid–T cell co-cultures to examine T-cell activation, degranulation, tumor killing, and responses to checkpoint blockade and various drugs.

- 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Chaoyuan Kuang:** Existing methods lack standardized, multimodal analysis of organoid–immune interactions; this protocol addresses that gap by integrating patient-derived co-cultures with complementary functional assays.

- 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Chaoyuan Kuang:** The protocol enables researchers to measure tumor–immune interactions, T-cell activation and cytotoxicity, tumor-cell apoptosis, cytokine secretion, and responses to therapeutic interventions.

- 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.4. **Nityanand Srivastava**: Yes. The procedure supports downstream molecular characterization, drug perturbation studies, immune profiling, flow cytometry, ELISpot, confocal microscopy, and live-cell imaging.

- 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Chaoyuan Kuang**: Future studies will be comparing immune cells and co-culture models, testing immunotherapies and combinations, and identifying biomarkers, resistance mechanisms, and clinical correlations.

- 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

What problem in your field of research does your JoVE article address?

- 1.6. **Chaoyuan Kuang, Assistant Professor**: (authors will present their testimonial statements live)

How does the video format of your article help you to share your work with the broader scientific community?

- 1.7. **Nityanand Srivastava/Mahshid Mohammadi, Staff Scientist/ Postdoctoral Fellow**: (authors will present their testimonial statements live)

Ethics Title Card

This research has been approved by the Institutional Review Board (IRB) at Montefiore Einstein Comprehensive Cancer Center Biobank

Protocol

Videographer's NOTE: Author provided narration of each step on audio channels 3&4.

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. Establishment of Tumor Organoids and Isolation of PBMCs

Demonstrator: Chaoyuan Kuang

- 2.1. To begin, wash freshly obtained tumor tissue, mince into small fragments [1]. Enzymatically or mechanically dissociate tissue into crypts or single cells while monitoring the reaction microscopically to check whether adequate dissociation is achieved [2]. After washing, collect the dissociated cells by centrifugation [3]. Then, dispense a mixture of cells and ice-cold basement membrane matrix or Matrigel as domes into pre-warmed 24-well culture plates [4]. Overlay solidified domes with complete patient-derived organoid medium containing ROCK (*rock*) inhibitor and Primocin [5 - TXT].

- 2.1.1. WIDE: Talent sitting at the biosafety cabinet cutting washed tumor tissue into small pieces

Videographer's NOTE: 2.1.1B - added a step- Author was going to provide text for this shot.

2.1.2 use clip C112_C632_0730BB_001

Author's Response: The dissociated organoids should be examined under the microscope to confirm the presence of single cells.

NOTE to video editor: Both videographer's and author's notes are not clear. Please use 2.1.1B added shot and 2.1.2 together. Most likely they are performing step 2.1.2 of dissociating the cells by pipetting or adding something to the cells and viewing under a microscope. Please contact PS integrator or the writer if any doubts.

- 2.1.2. SCOPE: Shot of talent monitoring tumor tissue dissociation into single cells under the microscope.

Videographer's NOTE: In addition to scope steps filmed on camera, the authors provided high resolution still images of each scope shot, use them as insets if required.

- 2.1.3. Shot of talent removing the tube from the centrifuge containing cell pellet.

Videographer: Obtain CU shot of cell pellet as well to show as an inset.

- 2.1.4. Shot of talent dispensing a mixture of cells and Matrigel in 24-well culture plates in the form of domes.

- 2.1.5. CU: Talent overlaying Matrigel domes with organoid growth medium supplemented with inhibitor and antibiotics.

TXT- Dilution ratio:

Rock inhibitor - 1:1000

Primocin antibiotic- 1:500 *Video editor: 1:500 and 1:1000 are ratios.*

2.2. Isolate peripheral blood mononuclear cells or PBMCs (*P-B-M-Sees*) from fresh human blood by Histopaque/Ficoll density gradient centrifugation [1] and harvest the PBMC-enriched buffy coat [2]. Wash the isolated PBMCs with 10 milliliters of PBS containing 0.1% Bovine serum albumin, by centrifugation at 1000 g for 7 minutes at room temperature, to remove Ficoll and residual platelets [3]. If not used immediately, add the isolated PBMCs at five to ten million cells per milliliter to 90% FBS and 10% DMSO for gradual freezing and subsequent storage in liquid nitrogen [4].

2.2.1. Talent setting up the density gradient centrifugation.

2.2.2. Talent collecting the buffy coat layer containing PBMCs from the freshly centrifuged tube.

Videographer: Obtain CU shot of cell pellet as well to show as an inset

2.2.3. Shot of talent washing the isolated PBMCs by centrifugation to remove Ficoll and residual platelets.

Videographer's NOTE: 2.2.3 filmed as WS and CU

2.2.4. Talent slowly adding freezing medium to the washed cells.

2.2.5. ~~Shot of talent transferring PBMCs to liquid nitrogen rack for long term storage.~~

Videographer's NOTE: 2.2.5 step REMOVED authors will explain

Author's Clarification: It is a very simple step and researchers know it, so we did not shoot this.

NOTE: VO merged with the previous shot such that it doesn't call for an action

3. Organoid Isolation and PBMC Recovery

Demonstrator: Chaoyuan Kuang

3.1. Next, retrieve the 24-well plate containing the organoid cultures from the 37 degrees Celsius incubator [1]. Remove spent organoid culture medium [2] and add 400 microliters of pre-warmed cell recovery solution in each well of the 24-well plate to dissolve the Matrigel - extracellular matrix and release organoids [3].

3.1.1. Talent taking the 24-well organoid culture plate from the incubator to the biosafety cabinet.

Videographer's NOTE: 3.1.2 & 3.1.3 C112_use clip C651_0730UQ_001

- 3.1.2. CU: Talent discarding old culture medium from each well of the 24 well organoid culture plate
- 3.1.3. CU: Talent adding pre-warmed cell recovery solution of each well to release the organoids for Matrigel matrix.

3.2. Gently resuspend organoids with a wide-bore pipette tip [1] and incubate the plate for 15 to 20 minutes at room temperature until the matrix dissolves and organoids are released [2].

3.2.1. CU: Talent resuspending the released organoids with wide-bore pipette tip.

Videographer's NOTE: 3.2.1 Authored mentioned that a CU was not needed for this step.

Videographer's NOTE: 3.2.2 use clip C112_C650_0730TJ_001

3.2.2. Talent briefly incubating the plate at room temperature for matrix to dissolve completely.

3.3. Transfer the organoid suspension to a 1.5 milliliter collection tube [1]. In a pre-cooled centrifuge, pellet organoids at 500 g for 5 minutes [2] and discard supernatant [3]. Wash the organoids pellet with 1 milliliter of basal medium supplemented with HEPES (*hpes*), Glutamax, and antibiotics [4- TXT], and centrifuge again at 500 g for 5 minutes at 4 degrees Celsius to re-pellet [5].

3.3.1. Talent transferring organoid suspension to collection tube.

3.3.2. Talent placing the tube containing organoid suspension in the centrifuge.

Videographer's NOTE: 3.3.2 filmed both placing and removing tube.

3.3.3. Talent removing the tube from the centrifuge to discard the supernatant.

3.3.4. CU: Talent washing the organoid pellet by resuspending in supplemented basal medium by pipetting up and down.

TXT - HEPES 10 mM

Glutamax 2mM

PenStrep dilution 1:100 *Video editor: 1:100 is a ratio*

Videographer's NOTE: 3.3.4 use clips C112_C636_0730NT_001 & C112_C654_0730CH_001

Videographer's NOTE: 3.3.5 use clip C112_C632_0730BB_001

3.3.5. Talent placing the tube in the centrifuge.

Videographer: Please obtain multiple reusable takes of 3.3.5. as these will be used later in the protocol

- 3.4. Now, resuspend the pelleted organoid in 12 milliliters of complete culture medium in a 15-milliliter collection tube [1]. Add 2 milliliters to each well of a 6-well tissue-culture-treated plate [2]. Next, allow the organoids to recover [3] by incubating for 24 hours at 37 degrees Celsius with 5% carbon dioxide before further processing [4].

3.4.1. CU: Talent resuspending organoid pellet in 12 milliliters of complete culture medium.

Videographer's NOTE: 3.4.1 and 3.4.2 - use clips C112_C636_0730NT_001 & C112_C654_0730CH_001

3.4.2. CU: Talent adding 2 milliliters of organoid suspension to each well of a 6-well tissue-culture treated plate.

3.4.3. SCOPE: Clear shot of organoids in tissue culture plate.

3.4.4. Talent incubating the 6-well plate for 24 hours at 37 degrees Celsius to allow organoid recovery.

Videographer's NOTE: 3.4.4: reuse shot in section 3.1.1

- 3.5. In Parallel to the above steps, rapidly thaw cryopreserved PBMCs using standard practice [1] and immediately combine them with 500 microliters of pre-warmed T-cell expansion medium to minimize osmotic shock [2]. Centrifuge thawed PBMCs at 400 *g* for 5 minutes to pellet the cells [3]. Remove freezing medium [4] and resuspend the pellet into 500 microliters of T-cell expansion medium containing benzonase to reduce cell clumping and improve single-cell suspension quality [5-TXT].

3.5.1. Talent thawing frozen PBMCs vial rapidly in a 37 degrees Celsius water bath.

Videographer's NOTE: 3.5.1B Please add this shot after 3.5.1- the new shot is described as suspending and resuspending the organoid. Please contact author for most detailed wording

Author's response: Add both 3.5.1 and 3.5.1B for the first shot

3.5.2. CU: Talent immediately adding 500 microliters of pre-warmed T-cell expansion medium to the vial containing thawed cells.

3.5.3. *Reuse 3.3.5.*

3.5.4. Talent discarding freezing medium from the pelleted PBMCs.

Videographer's NOTE: 3.5.4 use clips C112_C636_0730NT_001 & C112_C654_0730CH_001

- 3.5.5. CU: Talent resuspending the cell pellet in 500 microliters T-cell expansion medium containing benzonase by pipetting up and down. **TXT: Benzonase dilution – 1:1000**

Video editor: 1:1000 is a ratio

Videographer: Please obtain multiple reusable takes of 3.5.5. as these will be used later in the protocol

Videographer's NOTE: 3.5.5 use clips C112_C636_0730NT_001& C112_C654_0730CH_001

- 3.6. Wash the cells by re-pelleting at 500 g for 5minutes **[1]** and re-suspend PBMCs pellet in fresh expansion medium **[2]**. Repeat centrifugation at 500 g for 5 minutes one more time **[3]** and then resuspend cell pellet in 500 microliters of T-cell culture medium supplemented with IL-2 (*i-i-two*) to support viability and recovery **[4-TXT]**.

3.6.1. *Reuse 3.3.5.*

3.6.2. *Reuse 3.5.5.*

3.6.3. *Reuse 3.3.5*

3.6.4. *Reuse 3.5.5.* **TXT: IL-2 – 500 IU/ML**

Videographer's NOTE: 3.6.1- 3.6.4 (whole 3.6 section) repeat using use clips C112_C636_0730NT_001 & C112_C654_0730CH_001

- 3.7. Finally, plate the PBMCs into a single well of the 24-well plate **[1]** and incubate for overnight recovery at 37 degrees Celsius with 5% carbon dioxide **[2]**. Check cells before co-culture **[3]**.

3.7.1. Talent pipetting the cell suspension into a single well of 24-well plate inside the biosafety cabinet.

3.7.2. Talent placing the 24-well plate containing PBMCs in the incubator.

3.7.3. SCOPE: Clear shot of recovered PBMCs in 24-well plate.

Videographer's NOTE: 3.7.3 use clip C112_C650_0730TJ_001

4. Pre-Co-Culture Preparation

Demonstrator: Chaoyuan Kuang

- 4.1. Prepare the 96-well co-culture plate at least 24 hours in advance by coating the wells using 50 microliters per well of co-stimulatory antibody like, anti-CD28 (*C-D-twenty-eight*) **[1-TXT]** and store the plate at 4 degrees Celsius until further use **[2]**.

4.1.1. Talent adding co-stimulatory antibody suspension to the wells of a U-bottom 96 well plate. **TXT: anti-CD28 concentration - 3µg/mL**

4.1.2. Talent placing the plate at 4-degree Celsius until further use.

4.2. Now add the organoids, growing in the 6-well culture plate, to a 15-milliliter conical tube [1], and pellet them in a centrifuge at 500 g for 5 minutes [2]. Remove the supernatant [3]. Then resuspend the pellet and all adherent cells in 3 milliliters of enzymatic dissociation reagent, TrypLE (*Trip-lee*), to recover the full cell population [4].

Added action/step in 4.2.1: 4.2.1A Pipetting from 6-cell plate to 15 ml tube

Videographer's NOTE: Added a pipetting step before 4.2.1 "Pipette from 6 cell plate to a 15 ml tube"

4.2.1. Talent placing the 15-milliliter tube containing organoids in the centrifuge.

Videographer's NOTE: 4.2.1 Use clip C112_C636_0730NT_001

4.2.2. Talent discarding the supernatant.

4.2.2 and 4.2.3 use clips C112_C654_0730CH_001

4.2.3. CU: Talent resuspending the organoid pellet and adherent cells, in 6 milliliters of enzymatic dissociation reagent.

4.3. Incubate the cells at 37 degrees Celsius with 5% carbon dioxide, while gently mixing the cells after 5 to 10 minutes of incubation [1]. Monitor microscopically, until most organoids have dissociated into single tumor cells, avoiding over-digestion [2]. Quench the dissociation reagent with two times its volume using PBS [3]. Now, pellet the tumor cells at 500 g for 5 minutes, in a pre-cooled centrifuge and discard supernatant as demonstrated [4]. Resuspend the tumor cell pellet in 1 milliliter of fresh T-cell culture medium for the next steps [5].

4.3.1. Shot of talent gently tapping the tube containing organoids and dissociation reagent, while standing near the 37 degrees Celsius incubator.

4.3.2. SCOPE: Shot of organoids dissociated into single cells.

4.3.3. Talent quenching the dissociation reaction with 6 milliliters of PBS.

4.3.4. Shot of talent removing tube from the centrifuge and discarding the supernatant from the pelleted tumor cells.

Videographer's NOTE: 4.3.4 use clip C112_C636_0730NT_001 & C112_C632_0730BB_001

4.3.5 Use clip C112_C636_0730NT_001

4.3.5. Talent adding fresh 1 milliliter T-cell culture medium to the tumor cell pellet to resuspend them.

- 4.4. Take a 10-microliter aliquot of the tumor-cell suspension [1] and mix it with 10 microliters of 0.4% trypan blue solution at a one is to one ratio [2]. Determine viable cell numbers using a hemocytometer [3].
- 4.4.1. CU: Talent adding ten microliters of tumor cell suspension to a microcentrifuge tube.
- 4.4.2. CU: Talent mixing equal volume of viability dye with the tumor cell suspension to create a one is to one mixture.
- 4.4.3. SCOPE: Shot of viable, dye-excluded cells after trypan blue staining.
- 4.5. Prepare PBMCs for co-culture as demonstrated previously [1]. Finally, resuspend pelleted PBMCs in 500 microliters of T-cell medium, supplemented with cytokine IL-2 and immunomodulatory antibodies like anti-PD-1 (*p-d-one*) [2-TXT].

Added shot : 4.5.1a Talent adding cells from 24 well plate to 15 ml tube.

NOTE: Use both 4.5.1a and then 4.5.1 for shot 4.5.1

Videographer's NOTE: Add shot 4.5.1a before 4.5.1 author explains in audio of this shot but this step picks up from step 3.7 and is adding from 24 well to 15ml tube.

use clip C112_C632_0730BB_001

Author's response: Adding/transferring the cells from 24 well plate to 15 ml tube is correct

- 4.5.1. Shot of talent preparing PBMCs by centrifugation.
- 4.5.2. Talent adding 500 microliters of supplemented T-cell medium to the PBMCs pellet and resuspending by pipetting up and down. **TXT: IL-2 – 500 IU/mL; PD-1 – 40µg/mL**

Videographer's NOTE: 4.5.2 Use clip C112_C636_0730NT_001

- 4.6. Finally, prepare a suspension of tumor cells and PBMCs in the optimal ratio, based on the experimental setup, in T-cell medium [1-TXT].
- 4.6.1. CU: Talent mixing tumor cell suspension with PBMCs by gently pipetting up and down. **TXT: Ratio of tumor cells to PBMCs – can range from 1:5 to 1:20.**

Video editor: 1:5 and 1:20 are ratios

5. PBMC–Organoid Co-Culture and Drug Response Assessment

Demonstrator: Mahshid Mohammadi

- 5.1. Discard liquid from each well of the coated co-culture plate to remove unbound antibody [1] and wash wells twice with 200 microliters of Dulbecco's PBS [2]. Now, add 200 microliters of the prepared mixture of tumor cells and PBMCs to each well [3] of the 96-well plate [4] and incubate at 37 degrees Celsius with 5% carbon dioxide [5].

- 5.1.1. CU: Talent discarding the antibody suspension from each well of the 96-well plate.
- 5.1.2. Talent washing wells with 200 microliters of DPBS by pipetting up and down.
- 5.1.3. CU: Talent adding 200 microliters of mixture of tumor cells and PBMCs to each well of the U-bottom 96 well plate.
- 5.1.4. SCOPE: Shot of tumor cells plus PBMCs in the co-culture plate.
- 5.1.5. Talent placing the co-culture plate in the 37 degrees Celsius incubator.

Videographer's NOTE: 5.1.5 use clip C112_C650_0730TJ_001

- 5.2. Using the co-culture system, determine PBMCs' response to tumor cells in the presence of checkpoint inhibitor or small-molecule treatment [1]. Include appropriate controls and replication [2]. Proceed to assess the co-culture responses and T-cell mediated tumor killing by using confocal microscopy, flow cytometry [3] and live cell imaging [5].

- 5.2.1. Shot of talent setting up the drug response assessment assay using co-culture plates.
- 5.2.2. TEXT ON A PLAIN BACKGROUND:
 - Biological Replicates – Multiple organoid lines and PBMC donors
 - Technical Replicates – Each condition tested in triplicates
 - Controls - Organoid only, PBMC only as vehicle or activated, organoid plus PBMCs as vehicle, organoid plus drug, and PBMC plus drug conditions

- 5.2.3. Shot of talent sitting at the computer in confocal microscopy room performing image analysis.

- 5.2.4. ~~Shot of talent loading sample into the flow cytometer for analysis.~~

Videographer's NOTE: 5.2.4 REMOVE, VO is merged

- 5.2.5. Shot of talent performing live cell imaging to monitor T cell-mediated tumor killing over time.

Videographer: Please film shot 5.2.3, 5.2.4 and 5.2.5 as these were specifically requested by authors.

Videographer's NOTE: 5.2.5: 2 shots filmed for this step

Results

6. Results

- 6.1. Co-culture functionality was assessed by confocal microscopy after organoids were cultured alone or with PBMCs for 24 hours [1]. Ki67 (*k-i-sixty-seven*) expression was detected only when organoids were cultured alone, indicating active proliferation [2], whereas organoids co-cultured with PBMCs exhibited cleaved caspase-3 expression, indicating apoptosis or cell death [3].

6.1.1. LAB MEDIA: Figure 3

6.1.2. LAB MEDIA: Figure 3 *Video Editor: Please highlight the panel labelled Ki67*

6.1.3. LAB MEDIA: Figure 3 *Video Editor: Please highlight the panel labelled Caspase*

- 6.2. Apoptosis in co-cultures was quantified using a flow cytometry assay, and it showed that approximately 92 to 95% of T cells remained viable across all treatment conditions, with very low apoptosis [1]. Tumor organoid viability was also measured across different treatment conditions, and it was observed that organoid viability ranged from about 58 to 70% [2], wherein the co-culture alone showed the lowest viability [3].

6.2.1. LAB MEDIA: Figure 4B *Video Editor: Please highlight bars for the section labelled 'viable'*

6.2.2. LAB MEDIA: Figure 4C *Video Editor: Please highlight all bars except "Co-culture only" bar.*

6.2.3. LAB MEDIA: Figure 4C *Video Editor: Please highlight the bar for "Co-culture only".*

1. Organoids

Pronunciation link: No confirmed link found

IPA: /'ɔrgə,nɔɪdz/

Phonetic Spelling: OR·guh·noydz

2. Enzymatically

Pronunciation link: <https://www.merriam-webster.com/dictionary/enzymatic>

- IPA: /,en-zə-'ma-ti-k(ə-)lē/
Phonetic Spelling: en·zuh·MAT·ih·kuh·lee
3. Dissociation
Pronunciation link: <https://www.merriam-webster.com/dictionary/dissociation>
IPA: /,dɪ,sousi'eɪʃən/, /,dɪ,souʃi'eɪʃən/
Phonetic Spelling: dis·oh·see·AY·shuhn, dis·oh·shee·AY·shuhn
4. Centrifugation
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifugation>
IPA: /,sentɹə'fju'geɪʃən/
Phonetic Spelling: sen·truh·fyoo·GAY·shuhn
5. Matrigel
Pronunciation link: No confirmed link found
IPA: /'meɪtri,dʒel/
Phonetic Spelling: MAY·trih·jel
6. Primocin
Pronunciation link: No confirmed link found
IPA: /'praɪməsɪn/
Phonetic Spelling: PRY·muh·sin
7. Mononuclear
Pronunciation link: <https://www.merriam-webster.com/dictionary/mononuclear>
IPA: /,mɑ:nou'nu:kliər/, /,mɑ:nou'nju:kliər/
Phonetic Spelling: mon·oh·NOO·klee·ur, mon·oh·NYOO·klee·ur
8. Histopaque
Pronunciation link: No confirmed link found
IPA: /'hɪstəʊ,pɛɪk/
Phonetic Spelling: HIS·toh·payk
9. Ficoll
Pronunciation link: No confirmed link found
IPA: /'faɪkɔ:l/
Phonetic Spelling: FY·kawl
10. Cryopreserved
Pronunciation link: <https://www.merriam-webster.com/dictionary/cryopreservation>
IPA: /,kraɪəʊprɪ'zɜːvd/
Phonetic Spelling: cry·oh·prih·ZURVD
11. Extracellular
Pronunciation link: <https://www.merriam-webster.com/dictionary/extracellular>
IPA: /,ɛkstrə'seljələr/
Phonetic Spelling: ek·struh·SEL·yuh·lur
12. HEPES
Pronunciation link: No confirmed link found
IPA: /'hi:pi:z/
Phonetic Spelling: HEE·peeZ
13. Glutamax
Pronunciation link: No confirmed link found

- IPA: /'glu:tə,mæks/
Phonetic Spelling: GLOO·tuh·maks
14. Osmotic
Pronunciation link: <https://www.merriam-webster.com/dictionary/osmotic>
IPA: /ɑ:z'mɑ:tɪk/, /ɑ:s'mɑ:tɪk/
Phonetic Spelling: oz·MAH·tik, oss·MAH·tik
15. Benzonase
Pronunciation link: No confirmed link found
IPA: /'benzou,neɪs/
Phonetic Spelling: BEN·zoh·nays
16. Co-stimulatory
Pronunciation link: No confirmed link found
IPA: /,koʊ'stɪmjələ,tɔ:ri/
Phonetic Spelling: koh·STIM·yuh·luh·tor·ee
17. Enzymatic
Pronunciation link: <https://www.merriam-webster.com/dictionary/enzymatic>
IPA: /,enzə'mætɪk/
Phonetic Spelling: en·zuh·MAT·ik
18. TrypLE
Pronunciation link: No confirmed link found
IPA: /'trɪpli:/
Phonetic Spelling: TRIP·lee
19. Aliquot
Pronunciation link: <https://www.merriam-webster.com/dictionary/aliquot>
IPA: /'ælə,kwɑ:t/, /'ælə,kwət/
Phonetic Spelling: AL·uh·kwot, AL·uh·kwuht
20. Trypan
Pronunciation link: No confirmed link found
IPA: /'trɪpæn/
Phonetic Spelling: TRIP·an
21. Hemocytometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/hemocytometer>
IPA: /,hi:məsɑ'tɑ:mətər/
Phonetic Spelling: hee·muh·sy·TAH·muh·tur
22. Cytokine
Pronunciation link: <https://www.merriam-webster.com/dictionary/cytokine>
IPA: /'saɪtə,kam/
Phonetic Spelling: SY·tuh·kyne
23. Immunomodulatory
Pronunciation link: <https://www.merriam-webster.com/dictionary/immunomodulator>
IPA: /,ɪmjənʊ'mɑ:dʒələtɔ:ri/, /ɪ,mju:nʊ'mɑ:dʒələtɔ:ri/
Phonetic Spelling: im·yuh·noh·MOD·juh·luh·tor·ee, ih·myoo·noh·MOD·juh·luh·tor·ee
24. Dulbecco's
Pronunciation link: No confirmed link found

IPA: /dʌl'bekoʊz/

Phonetic Spelling: dul·BEK·ohz

25. Confocal

Pronunciation link: <https://www.merriam-webster.com/dictionary/confocal>

IPA: /ˌkɑːn'foʊkəl/

Phonetic Spelling: kon·FOH·kuhl

26. Cytometry

Pronunciation link: No confirmed link found

IPA: /sai'tɑːmətri/

Phonetic Spelling: sy·TAH·muh·tree

27. Ki67

Pronunciation link: No confirmed link found

IPA: /ˌkeɪ aɪ ˌsɪksti 'seɪvən/

Phonetic Spelling: kay·eye·SIK·stee·SEV·uhn

28. Proliferation

Pronunciation link: <https://www.merriam-webster.com/dictionary/proliferation>

IPA: /prəˌlɪfə'reɪʃən/

Phonetic Spelling: pruh·lif·uh·RAY·shuhn

29. Caspase

Pronunciation link: No confirmed link found

IPA: /'kæsp.eɪs/

Phonetic Spelling: KAS·pays

30. Apoptosis

Pronunciation link: <https://www.merriam-webster.com/dictionary/apoptosis>

IPA: /ˌæpəp'toʊsɪs/, /ˌæpə'toʊsɪs/

Phonetic Spelling: ap·uhp·TOH·suhs, ap·uh·TOH·suhs