APOPTOSIS (ANTI-PS)

biocytometry kit

technical guide

sampling
HUMAN

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Contents

1. Introduction

Founded in 2016, Sampling Human is a life science tool company that believes every health decision starts with a single cell. Our mission is to weave single-cell precision into the fabric of our lives.

Sampling Human's biocytometry platform has made a scale-shifting breakthrough in single-cell analysis by programming samples to resolve themselves. Our bioparticles work in solution, evaluating every cell in a sample all at once. The resulting wealth of information makes cellular responses meaningful and actionable.

Biocytometry is driving the translation of a wealth of data into practical solutions in drug discovery, diagnostics, and medicine. Our platform is being adopted internationally in clinical facilities and leading universities. Any laboratory with a plate reader can use biocytometry to accelerate its research.

We cannot wait to work with you to build a world where cellular evidence drives our daily health decisions.

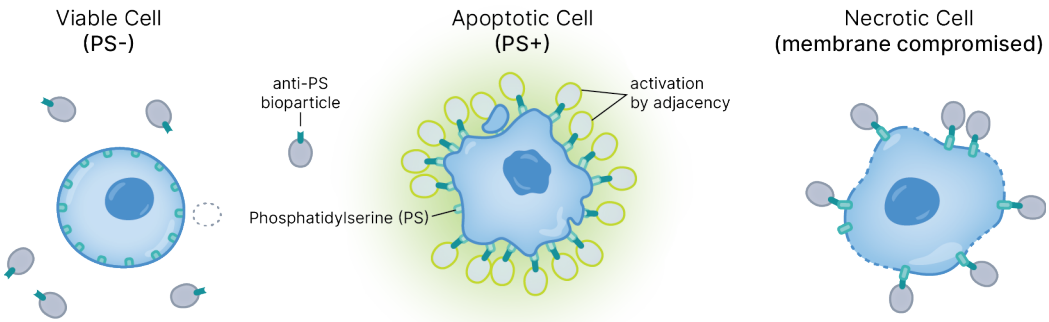
- Sampling Human Team

2. Technology

2.1 Overview

Each biocytometry kit contains bioparticles engineered to target a specific cell type, cell state, or cell interaction. When added to a sample, the bioparticles bind to targeted cells based on the surface markers they express. Bioparticles form smart capsules by completely surrounding cells of interest. Within each smart capsule, bioparticles are activated by adjacent bioparticles to evaluate the cell's immunoprofiles as a whole. Matching profiles result in the production of a luminescent reporter while incomplete profiles do not. The luminescent signal is measured on a plate reader, and subsequently converted to a cell count for enumeration.

The bioparticles of the Apoptosis (anti-PS) Biocytometry Kit target phosphatidylserine which is displayed on the outer leaflet of the cell membrane in the early stages of apoptosis. Viable cells maintain phosphatidylserine on the inner membrane leaflet where bioparticles are unable to bind. Necrotic cells and cell debris do not induce reporter production because bioparticles are unable to bind in sufficient density to trigger activation.



2.2 Example Data

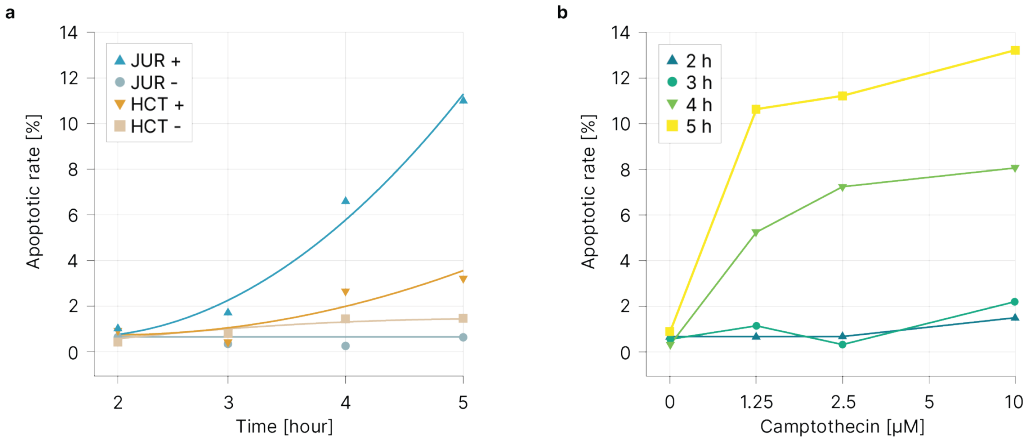


Figure 1. Apoptosis quantification in Jurkat and HaCaT cells following treatment with apoptogenic agents.

(**a**) Time-course analysis using HaCaT and Jurkat cell monocultures. Cells were seeded at a density of 2,500 cells/well in RPMI and treated with 0.25 mM DTT to induce apoptosis. Incubation proceeded at 37°C in a 5% CO2 atmosphere for intervals of 2, 3, 4, or 5 hours. Apoptosis was quantified using Apoptosis (anti-PS) Biocytometry Kit following established preparation protocol. Signals were normalized to the number of cells, established prior to the biocytometric analysis. Analysis reveals differential sensitivity of HaCaT and Jurkat cell lines to DTT treatment. (**b**) Concentration-response curves for Jurkat cell line and camptothecin. Cells were seeded at a density of 2,500 cells/well in RPMI supplemented with 10% FBS and treated with varying concentrations of camptothecin (0 - 10 µM). Incubation proceeded at 37°C in a 5% CO2 atmosphere for intervals of 2, 3, 4, or 5 hours. Apoptosis was quantified using Apoptosis (anti-PS) Biocytometry Kit following established preparation protocol. Signals were normalized to the number of cells, established prior to the biocytometric analysis. Notable onset of apoptosis is observed at the 3-hour mark.

3. Product Components and Storage

Item	Quantity (pc)	Volume	Cat. #
Bioparticles (anti-PS)	1	lyophilized reagent	**SH2100**
Rehydration Buffer	1	110 µl	**SH0411**
Resuspension Buffer	1	1500 µl	**SH0412**
Gel Medium	10	750 µl	**SH0203**
4X Luminescence Buffer	1	1500 µl	**SH0811**
Luminescence Substrate	1	lyophilized reagent	**SH0812**
Positive Control	1	lyophilized reagent	**SH0701**

Storage Conditions

Store the kit with all contents at -20°C, protected from light. See the product label for expiration date.

4. Materials to be Supplied by the Users

4.1 Equipment

Various instruments, irrespective of their manufacturer or model, can be used to perform the biocytometry protocol, provided they meet the necessary technical specifications.

Equipment	Specifications
Heat block / Water bath / Incubator	Capable of maintaining 37 °C
Vortex	500-3000 RPM
Centrifuge	Fixed angle Holds 1.7 ml tubes Capable of 200 ×*g*
Thermoshaker	Holds 1.7 ml tubes Capable of 1400 RPM Capable of maintaining 30-37 °C
Refrigerator	Capable of maintaining 4 °C
Incubator	Capable of maintaining 30 °C
Plate reader	With luminescence readout functionality ≤ 100 amol ATP sensitivity

4.2 Consumables

Examples of suitable products are listed below. Items different from those listed can be used, provided they meet the same technical specifications.

Item	Example products
Luminescence-grade 96-well white plate	• Falcon® 96-well White Flat Bottom TC-treated Microtest Assay Microplate • Pierce™ 96-Well Polystyrene Plates, White Opaque • Nunc™ 96-Well Polypropylene Sample Processing & Storage Microplates
1.7 ml microcentrifuge tube	• ClickSeal™ Microcentrifuge Tubes, Graduated, National Scientific Supply • Eppendorf Safe-Lock Tubes • Corning® Costar® microcentrifuge tubes with snap cap

5. Specifications

5.1 Kit Components

Bioparticles (Anti-PS) are the active component of the biocytometry assay. They are supplied in a dried form and rehydrated with the addition of **Rehydration Buffer**. Each tube of **Bioparticles** contains enough reagent for 10 reactions (8 samples, 1 positive control, 1 negative control).

Rehydration Buffer is used for the rehydration of the **Bioparticles.** Upon rehydration, **Bioparticles** should be used immediately.

Resuspension Buffer is used for the resuspension of samples as well as the positive and negative controls. It is not recommended to store cells in **Resuspension Buffer** for extended periods. Only transfer cells to **Resuspension Buffer** when you are ready to begin your assay. If storage is necessary, place cells at 4 °C.

Gel Medium is a semi-permeable nutritive hydrogel with thermoresponsive properties. The **Gel Medium** must be pre-heated to 37 °C for at least 10 minutes prior to use. There is roughly 10 minutes of handleable working time after being removed from heat before the **Gel Medium** solidifies at room temperature.

4X Luminescence Buffer is provided in a concentrated form and must be diluted prior to use. The diluted form will remain stable at room temperature and can be prepared ahead of time.

Luminescence Substrate is provided in lyophilized form to be rehydrated in **1X Luminescence Buffer** to be used as **Readout Reagent**. The **Luminescence Substrate** is light sensitive in both its dried and rehydrated form. Once rehydrated, the **Readout Reagent** should be used immediately.

Positive Control is supplied as a lyophilized reagent to be rehydrated in **Resuspension Buffer**. Once rehydrated, the **Positive Control** is treated in the same way as a normal sample. At readout, the **Positive Control** provides a strong signal for calibration and indicator of the activity of the **Bioparticles**.

5.2 Sample

Sample Composition

The biocytometry kit is currently compatible with PBMCs, cryoperserved samples, primary cell cultures, organoids, and immortalized cell lines. Cells should be pelleted and resuspended in the provided **Resuspension Buffer** prior to use. Samples should not exceed given limits (assuming use of a standard reaction size):

Sample volume: 100 µl

Total number of cells: 10,000

Target cells

The Apoptosis (anti-PS) Biocytometry Kit identifies target cells that display phosphatidylserine on the outer leaflet of the membrane and whose membrane is intact. Such phenotype is a common predictor of early stage of apoptosis. Necrotic cells are not quantified.

5.3 Readout

Labware Definition

Users must supply their own labware for readout. Please refer to the manufacturer guidelines of the 96-well plate and the instrument recommended settings.

Plate Reader

Our kits have been validated for use with plate readers with luminescence readout sensitivity greater than 1000 amol. This criterion is met by the majority of multimode plate readers available. For guidance on configuring different plate reader models, we encourage you to reach out to our application support team.

Recommended read settings:

Detection Method: Luminescence
Optics Type: Luminescence Fiber/Filter
Read Type: Endpoint/Kinetic
Interval: 1 minute or minimum possible
Integration Time: 1 s
Gain: 255
Read From: Top
Lid: Off
Read Height: Default (according to plate manufacturer)
Plate Type: 96-well white plate (according to plate manufacturer)
Number of reads: 5 (use average as final readout values)
Attenuation: None
Settle Time: 0 ms

*Note: Not all of these settings may be available or necessary depending on the make and model of your instrument

6. Assay Preparation

6.1 Overview

The biocytometry protocol is optimized for ease of use and efficiency. The protocol consists of three basic steps: 1) sample preparation, 2) biocytometry assay and 3) luminescence readout. Less than 30 minutes total handling time is expected, with a hands-off incubation period of 5.5 hours. All steps in the protocol are performed at room temperature unless specified otherwise.

6.2 Notes

- Please read the entire protocol to become familiar with the components and the assay procedure.
- A single kit is for the analysis of a maximum of 10 reactions at one time (8 samples, 1 positive control, and 1 negative control).

  ⚠ **For first time users:** it is recommended to first perform one assay using only the positive control and negative control as samples.

- Lyophilized reagents may be hard to see, but are present in the tip of the tube.
- Store components at -20 °C until required for use.
- Allow the kit to equilibrate to room temperature for 5 minutes before beginning the protocol.
- Briefly centrifuge all kit components after thawing (100 x*g*, 5 sec) to facilitate maximum recovery.

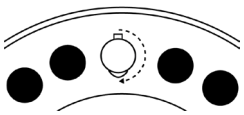
6.3 Biocytometry Protocol

Sample Preparation

1. Preheat a water bath or heat block to 37 °C.
2. Place **Resuspension Buffer** at 37 °C to thaw (roughly 5 min).
3. Harvest cells (attachment-dependent or attachment-independent) and resuspend the cell pellet to a concentration of 100,000 cells/ml in **Resuspension Buffer.**
4. For each **Sample**, transfer 100 µl of cell suspension into a new microcentrifuge tube and label accordingly.
5. Rehydrate **Positive Control** in 200 µl of **Resuspension Buffer** and incubate at room temperature for 5 minutes. Vortex thoroughly after incubation.
6. Transfer 100 µl of rehydrated **Positive Control** to a new microcentrifuge tube and label accordingly
7. As a **Negative Control**, transfer 100 µl of **Resuspension Buffer** to a new microcentrifuge tube and label accordingly

Biocytometry Assay

8. For each reaction (including **Positive** and **Negative Controls**), place one tube of **Gel Medium** at 37 °C and hold until further use.
9. Rehydrate **Bioparticles (anti-PS)** in 60 µl of **Rehydration Buffer** and incubate at room temperature for 5 minutes. Vortex gently after incubation to obtain a homogeneous suspension.
10. Transfer 5 µl of **Bioparticles (anti-PS)** to each reaction for analysis.
11. Gently vortex each reaction.
12. Place the tubes into a fixed-angle centrifuge, orienting the hinge away from the center, and spin down at 200 x g for 1 minute.
13. Without removing the tubes from the centrifuge, twist the tubes 180° until the hinge faces inwards to the center.



14. Spin down 4 more times in the same manner, rotating the tube 180° between each spin down. A small pellet should be visible.
15. ⚠ *Gently* pipette up and down until the pellet is dispersed.
16. Place the reactions on a thermoshaker and resuspend at 1400 RPM, 30° C for 1 minute.

17. Retrieve **Gel Medium** tubes from 37 °C.
18. Transfer 100 µl from each reaction into the corresponding **Gel Medium** tube. Once all transfers are complete, place the tubes in a rack and invert the entire rack 10 times to ensure uniform mixing.

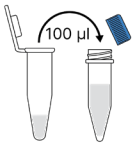
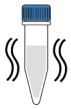
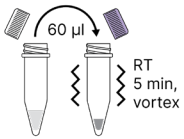
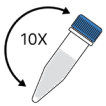
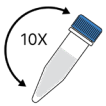
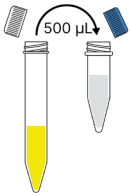
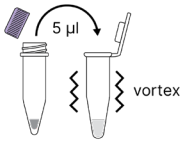
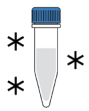
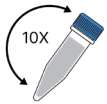
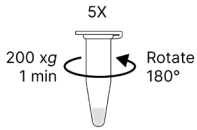
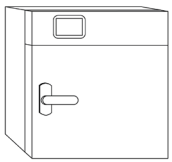
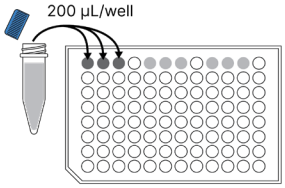
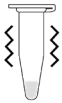
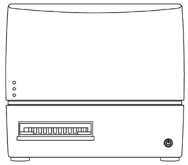
    ⚠ The **Gel Medium** should be fully liquid and should move when the tube is inverted
19. Incubate the reactions at 37 °C for 5 minutes.

    ⚠ Exceeding the incubation time at this step may compromise assay performance.
20. Retrieve the reactions and invert 10 times again.
21. Incubate the reactions at 4 °C for 10 minutes to set the hydrogel.
22. Transfer the reactions to an incubator at 30 °C for 5.5 hours to facilitate the bioprocessing.

Luminescence Readout

23. Preheat a thermoshaker to 37 °C.
24. Retrieve a white 96-well plate.
25. Transfer the reactions from 30 °C incubation to the preheated thermoshaker to incubate for 10 minutes at 37 °C.
26. While the reactions incubate, prepare the Readout Reagent by transferring the entire contents of the **4X Luminescence Buffer** to 4.5 ml of ddH_2O.
27. Rehydrate the **Luminescence Substrate** by adding 1 ml of **1X Luminescence Buffer**.
28. Vortex vigorously to dissolve completely.
29. Transfer the resuspended **Luminescence Substrate** back to the remaining **Luminescence Buffer** to yield 6 ml of **Readout Reagent**.
30. Once the incubation of the reactions is complete, add 500 µl of the Readout Reagent to each tube. The remaining Readout Reagent can be discarded.
31. After transferring all reactions, place the tubes in a rack and invert the entire rack 10 times to ensure uniform mixing.
32. For each reaction, dispense 200 µl per well in replicates of at least 3 in a white 96-well plate. To minimize signal spillover, leave a blank well between different samples.
33. Place the plate in the microplate reader and initiate the readout program (See **Section 5.3 Readout** for details).

7. Picture Protocol

Reaction	Incubation	Readout
Preheat **Gel Medium** 37 °C hold 	Transfer reaction to **Gel Medium** 	Melt reaction 37 °C 5 min 
Add **Rehydration Buffer** to **Bioparticles** 	Invert reaction, incubate, invert again  37°C 5 min  	Add **Readout Reagent*** to reaction 
Add **Bioparticles** to **Sample** 	Chill reaction 4°C 10 min 	Invert to mix 
Spin down and rotate 5X 	Incubate at 30 °C for 5.5 hr 	Dispense to 96-well plate 
Resuspend on shaker 1400 RPM 30 °C 1 min 		Readout on plate reader 

*__Readout Reagent__ is provided in separate components, **4X Luminescence Buffer** and **Luminescence Substrate**

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