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ab270782 Serine Protease Assay Kit (FAM-Phe-DAP)

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Serine Protease Assay Kit (FAM-Phe-DAP) datasheet:

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For the detection of intracellular chymotrypsin-like serine protease activity *in vitro*.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

These Serine Protease Assay Kits enable researchers to detect intracellular chymotrypsin-like serine protease activity *in vitro* without lysing the cell.

The fluorescent inhibitor reagents provided in these kits are non-cytotoxic green or red fluorescent inhibitors that covalently bind with active serine protease enzymes. This approach is a powerful method to assess the intracellular levels of chymotrypsin- like serine protease activity in vitro. Just add the fluorescent inhibitor reagent directly to the cell culture media, incubate, and wash. Because these inhibitors are cell-permeant, they will efficiently diffuse in and out of all living cells. If there is an active chymotrypsin-like enzyme inside the cell, it will covalently bind with the fluorescent inhibitor reagent and retain the green or red fluorescent signal within the cell. Cells containing lower concentrations of chymotrypsin-like enzyme activity will retain a lower level of fluorescence compared to cells containing higher concentrations of this effector enzyme. There is no interference from pro-enzymes nor inactive forms of the enzymes. If the treatment is activating chymotrypsin-like serine proteases, positive cells will fluoresce brighter than the normal baseline negative cells, thus enabling researchers to clearly differentiate the populations.

2. Materials Supplied and Storage

Store Green FAM-Phe-DAP fluorescent inhibitor reagent at -20°C upon receipt.

Store the rest of the kit at +4°C.

Δ Note: Once reconstituted with DMSO, use Green FAM-Phe-DAP inhibitor reagent immediately, or store at ≤-20°C for up to 6 months, protected from light. Avoid repeated freeze/thaw cycles.

Item	Quantity		Storage temperature
	25 tests	100 tests	
10X Cellular Wash Buffer	1 x 15 ml	1 x 60 ml	+4°C
Fixative	1 x 6 ml	1 x 6 ml	+4°C
Hoechst 33342, 200 µg/mL	1 vial	1 vial	+4°C
Propidium Iodide, 250 µg/mL	1 x 1 mL	1 x 1 mL	+4°C
Green FAM-Phe-DAP fluorescent inhibitor reagent	1 vial	4 vials	-20°C

3. Materials Required, Not Supplied

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

- DMSO, 50 µL per vial to reconstitute inhibitor reagent
- DiH_2O, 135-540 mL to dilute 10X Cellular Wash Buffer
- Phosphate buffered saline (PBS) pH 7.4, up to 100 mL, to dilute inhibitor reagent and handle cells
- FBS and/or BSA to add to the buffer when handling cells
- Cultured cells treated with the experimental conditions ready to be labeled
- Reagents to stimulate serine protease activity or induce apoptosis, such as staurosporine or camptothecin
- 90% EtOH or 3% formaldehyde to create live/dead controls for Propidium
- Iodide staining
- Black 96-well microtiter plate, flat bottom, non-treated, non-sterile. If using a bottom reading instrument, use a plate with black walls and a clear bottom. If culturing cells in the plate, use a sterile black tissue culture plate.
- Hemocytometer
- Centrifuge at 200 *x g*
- 15 mL polystyrene centrifuge tubes (1 per sample)
- Fluorescence microscope
- Fluorescence plate reader
- Flow Cytometer

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Experimental Preparation

5.1 Staining cells with fluorescent inhibitor reagent can be completed within a few hours. However, fluorescent inhibitor reagent is used with living cells, which require periodic maintenance and cultivation several days in advance. In addition, once the proper number of cells has been cultivated, time must be allotted for the experimental treatment or control process which may require additional incubation. Create cell populations, such as:

5.1.1 Cells that were exposed to the experimental condition, treatment, or stimulation.

5.1.2 Normal non-treated cells of the same cell line or type that were not exposed to the experimental condition that will act as baseline controls of serine protease enzyme activity.

5.2 As fluorescent inhibitor reagent detects serine protease activity, plan the experiment such that fluorescent inhibitor reagent will be diluted and administered at the time when elevated serine protease activity is expected to be evident in the cell treatment population. The recommended volume of 50X fluorescent inhibitor reagent is 10 µL per 490 µL of cells at 5 x 10^5 cells/mL, but the amount may vary based on the experimental conditions and the instrument used for analysis. Each investigator should adjust the amount of fluorescent inhibitor reagent to accommodate the particular cell line and research conditions.

5.3 Culture cells to a density optimal for the specific experiment or control protocol. Cell density should not exceed 10^6 cells/mL. Cells cultivated in excess of this concentration may begin to naturally enter apoptosis. An initial experiment may be necessary to determine when and how much fluorescent inhibitor reagent to use as the resulting positive signal is a direct measurement of chymotrypsin- like enzyme activity occurring during the incubation period.

6. Controls

Establishment of positive and negative cell population controls to assess constitutively expressed cellular housekeeping serine proteases can be somewhat challenging. As discussed in Section 5, a normal, non-stimulated cell population control is needed to act as the reference baseline of enzymatic activity. Also, in some cell lines, the addition of conventional apoptosis inducing agents such as camptothecin or staurosporine (Section 7) can generate elevated levels of subsets of the larger chymotrypsin family of serine protease enzymes.

Create experimental samples and control cell populations:

- Treated experimental population(s): cells exposed to the experimental condition(s).

- Negative control: non-treated cells grown in a normal cell culture environment.

- Positive control: cells induced to activate serine proteases using a known activation protocol.

The induced positive cell population and negative control cell population tubes should come from a common pool of cells and contain similar quantities of cells. Create negative controls by culturing an equal volume of non-induced cells for every labeling condition. For example, if labeling with FLISP and analyzing samples by flow cytometry, create 4 populations:

Control #	
1 and 2	Unlabeled, induced and non-induced populations.
3 and 4	Fluorescent inhibitor reagent-labeled: induced and non-induced.

If using FAM-fluorescent inhibitor reagent with Propidium Iodide (PI) vital dye for dual staining, create PI instrument controls using formaldehyde or EtOH to compensate for bleed-over of the PI signal into FAM-fluorescent inhibitor reagent signal.

7. Stimulate serine protease activity

Determine a reproducible method for stimulating serine protease activity to obtain a positive control prior to commencing the experiment. Induction of apoptosis may trigger serine protease activity as they are involved in protein degradation along with caspase enzymes. For example, apoptosis may be induced with:

- 2-4 µg/mL camptothecin for >4 hours.
- 1-2 µM staurosporine for >4 hours.

8. Preparation of Green FAM-Phe-DAP inhibitor reagent

– Fluorescent inhibitor reagent is supplied as a lyophilized powder that may be slightly visible as an iridescent sheen inside the vial. Protect from light and use gloves when handling. Because the 50X fluorescent inhibitor reagent staining solution must be used immediately, prepare it just before staining.

8.1 Reconstitute each vial of fluorescent inhibitor reagent with 50 µL DMSO to form the 250X stock concentrate. The FAM-fluorescent inhibitor reagent stock concentrate should be colorless or light yellow; the SR101-fluorescent inhibitor reagent stock concentrate should be pink or red. Once reconstituted, the stock concentrate may be stored at ≤-20°C for 6 months protected from light and thawed no more than twice during that time.

8.2 Immediately prior to addition to the samples and controls, dilute the 250X stock concentrate 1/5 by adding 200 µL PBS to each vial to form the 50X fluorescent inhibitor reagent staining solution. Use the 50X staining solution within 15 mins of dilution into aqueous buffers as the CMK and DAP reactive groups are water labile.

9. Preparation of 1X Cellular Wash Buffer

10X Cellular Wash Buffer is an isotonic solution used to wash cells following exposure to fluorescent inhibitor reagent. It contains mammalian proteins to stabilize cells stained with fluorescent inhibitor reagent and sodium azide to retard contamination (1X Cellular Wash Buffer contains 0.01% w/v sodium azide). Cell media containing FBS and other additives may be used instead of 1X Cellular Wash Buffer.

- 10X Cellular Wash Buffer may form precipitates during cold storage. If this happens, gently warm it until all crystals have dissolved. Do not boil.

- Dilute 10X Cellular Wash Buffer 1/10 in diH_2O. For example, add 15 mL 10X Cellular Wash Buffer to 135 mL diH_2O for a total of 150 mL.

Δ Note: 1X Cellular Wash Buffer may be stored at 4°C and used within 1 week or frozen and used within 6 months.

10. Propidium Iodide

Propidium Iodide (PI) is used to distinguish between living and dead cells by staining necrotic, dead, and membrane-compromised cells red. PI is an intercalating fluorescent reagent that stoichiometrically binds every four to five base pairs of DNA. PI is membrane impermeant, which prevents it from reaching the DNA in viable cells, thus allowing the identification of dead cells in a population.

Upon binding to DNA, the fluorescence intensity potential of PI is enhanced 20-30-fold. Nucleic acid-bound PI has a red-shifted absorbance/excitation maximum of 535 nm and an emission maximum of 617 nm. PI efficiently excites at 488-492 nm. Its excitation and emission spectra allow for efficient analysis using fluorescence microscopy or flow cytometry.

All Green FAM-fluorescent inhibitor reagent kits include PI in a soluble, ready to use formulation (1 mL at 250 µg/mL). PI is not included in the red SR101-fluorescent inhibitor reagent kits due to the overlap in emissions.

If analyzing the cells using flow cytometry, establish a PI-positive control population of dead cells to assist with proper compensation. For example, create PI-positive controls using formaldehyde or EtOH to kill cells. A stimulation or apoptosis induction method is not as effective as a solvent treatment (like EtOH) at creating PI-positive cell controls for instrument calibration.

To set up PI instrument controls:

10.1 Label 2 centrifuge tubes.

10.1.1 PI-negative (live).

10.1.2 PI-positive (dead).

10.2 Add 1-5 x 10^5 non-stimulated live healthy cells to each tube.

10.3 Centrifuge at 200 *x g* for 5-10 mins at room temperature (RT) to pellet cells; remove supernatants.

10.4 To create the PI-negative control (live cells), resuspend the cells in 300 µL of PBS to maintain the integrity of the cell membrane. Gently vortex for 30 secs and then wash cells (Steps 10.6-10.8).

10.5 To create the PI-positive control (membrane-compromised dying cells): Use formaldehyde: Resuspend cells in 300 µL 3% v/v formaldehyde (in 97% PBS). Incubate 30 mins on ice and then wash cells (Steps 10.6-10.8).

OR use EtOH: Resuspend cells in 300 µL 90% EtOH (in 10% PBS). Gently vortex for 30 secs and then wash cells (Steps 10.6-10.8).

10.6 Add 1 mL PBS.

10.7 Centrifuge at 200 *x g* for 5-10 mins; remove supernatants.

10.8 Resuspend in 600 µL PBS+1% BSA.

10.9 Add 3 µL PI to both tubes. If different volumes were used, add PI at 0.5% v/v.

10.10 Incubate for 5-10 mins.

10.11 Read immediately on the flow cytometer and use to compensate bleed over of the PI signal into the FAM channel.

11. Hoechst 33342

- Hoechst 33342 is a cell-permeant nuclear stain that emits blue fluorescence when bound to double stranded DNA. It is used to stain the nuclei of living or fixed cells, to distinguish condensed pyknotic nuclei in apoptotic cells, and for cell cycle studies.
- It is provided ready to use at 200 µg/mL and can be used with FAM-fluorescent inhibitor reagent, SR101-fluorescent inhibitor reagent, and PI to label cell nuclei.
- When bound to nucleic acids, it has a maximum absorbance at 350 nm and a maximum emission at 480 nm. It is revealed under a microscope using a UV-filter with excitation at 365 nm and emission at 480 nm.

12. Fixative

- The Fixative is a formaldehyde solution designed to cross-link and aggregate intracellular components. If the stained cell populations cannot be evaluated immediately after labeling with FLISP, add Fixative at a ratio of 1:5-1:10. For example, to use Fixative at 1:10, add 100 µL Fixative to 900 µL cells. Never add Fixative until all the staining and final wash steps have been completed. Fixed cells may be stored protected from light on ice or at 2-8°C for up to 16 hours.
- The Fixative will not interfere with the carboxyfluorescein (FAM) or sulforhodamine-101 (SR101) label. Do not use absolute ethanol- or methanol- based fixatives as they will inactivate the FAM-fluorescent inhibitor reagent or SR101-fluorescent inhibitor reagent labels. Do not fix cells that will be stained later with Propidium Iodide.

13. Staining protocol for suspension cells

- Prepare experimental and control cell populations. Ideally, cell concentration should be 3-5 x 10^5 cells/mL. The concentration should not exceed 10^6 cells/mL, as cells cultivated in excess of this concentration may begin to naturally enter apoptosis.
- Just prior to staining with fluorescent inhibitor reagent, cells may need to be concentrated to 2-5 x 10^6 cells/mL as both microscopy and plate reader analysis methods require high cell concentrations. Start with a larger volume of cells at 3-5 x 10^5 cells/mL (which is a typical density for cell culture) and then concentrate cells and resuspend to 500 µL per sample when ready for fluorescent inhibitor reagent staining.

13.1 Expose cells to the experimental or control condition. If analyzing with a flow cytometer, set aside the appropriate single-color cell populations to create instrument controls (fluorescent inhibitor reagent-induced and fluorescent inhibitor reagent-non-induced cells, and if necessary, PI-positive and PI-negative cells).

13.2 If analyzing with a fluorescence microscope or plate reader, concentrate cells to 2-5 x 10^6 cells/mL just prior to fluorescent inhibitor reagent staining. Fluorescence microscopy requires an excess of 2 x 10^6 cells/mL to obtain 5-20 cells per image field. Flow cytometry can analyze samples at 3-5 x 10^5 cells/mL.

13.3 Transfer 490 µL cells into fresh tubes.

13.4 Add 10 µL 50X Green FAM-Phe-DAP inhibitor reagent staining solution and gently mix. If different cell volumes were used, add 50X fluorescent inhibitor reagent staining solution at a ratio of 1:50. Mix by gently flicking the tubes. The amount of fluorescent inhibitor reagent should be optimized for each cell line and experimental condition.

13.5 Incubate cells at 37°C protected from light. The incubation period may range from 30 mins to several hours and should be optimized for each cell line and experimental condition. As cells may settle on the bottom of the tubes, gently resuspend by swirling cells every 10- 20 mins to ensure an even distribution of fluorescent inhibitor reagent.

13.6 If cells are to be analyzed with a microscope, cells may be dually stained with Hoechst. Add Hoechst at 0.5% v/v and incubate 10-20 mins at RT or 37°C. For example, if the cell

suspension is at 500 µL, add 2.5 µL Hoechst. Omit this step if cells are to be analyzed by flow cytometry or fluorescence plate reader.

13.7 Add 2 mL 1X Cellular Wash Buffer and gently mix.

13.8 Centrifuge at 200 *x g* for 5-10 mins at RT.

13.9 Carefully remove and discard supernatants. Gently vortex the pellets to disrupt clumping. Resuspend in 1 mL 1X Cellular Wash Buffer and gently mix.

13.10 Centrifuge cells at 200 *x g* for 5-10 mins at RT.

13.11 Carefully remove and discard supernatants. If analyzing by fluorescence microscopy or fluorescence plate reader, repeat wash process a third time. If using a flow cytometer, two wash steps are generally sufficient.

13.12 Gently vortex pellets to disrupt clumping.

13.13 Read cells within 4 hrs or fix. Fixed cells may be stored protected from light on ice or at 2-8°C for up to 16 hrs.

- If analyzing with a fluorescence microscope, go to Section 15.
- If using a flow cytometer, go to Section 16.
- If using a fluorescence plate reader, go to Section 17.

14. Staining protocol for adherent cells

Adherent cells need to be handled carefully to avoid the loss of any cells that round up and come off the culture surface. In microscopy or plate reader applications where trypsinization is not required, adherent cells can be stained and washed directly on the chamber slide, well, or culture surface. To avoid losing cells that are no longer adherent during washing, spin down all overlay media and wash buffer and recombine washed cell pellets with the adherent samples prior to analysis.

Cells may be trypsinized to create suspensions, which may be labeled with Green FAM-Phe-DAP inhibitor reagent before or after trypsinization. Do NOT trypsinize cells prior to labeling with a live/dead stain, like PI or 7-AAD. Cell membranes exposed to trypsin could be transiently permeable to live/dead dyes for a variable time depending upon the cell line.

14.1 Culture cells in T25 flasks, culture dishes, or chamber slides and expose to the experimental or control conditions.

14.2 If suspension cells are required for the final analysis, go to Step 14.3. If staining cells while adherent, go to Step 14.5.

14.3 Trypsinize adherent cells:

14.3.1 Remove overlay media. Spin to pellet loose cells.

14.3.2 Trypsinize adherent cells. Alternatively, fluorescent inhibitor reagent labeling can be performed first, followed by washing and trypsinization steps.

14.3.3 Neutralize with 2-5 mL of a trypsin inhibitor, such as cell culture media containing 20% FBS.

14.4 Adjust volume of trypsinized cell samples for staining:

14.4.1 Centrifuge at 200 *x g* for 5-10 mins at RT.

14.4.2 Remove all but ~100 µL supernatant.

14.4.3 Resuspend cells in 500 µL cell culture media containing 10-20% FBS.

14.4.4 If necessary, count cells and adjust the concentration and volume of cell suspension to fit the experiment.

14.5 Add the 50X fluorescent inhibitor reagent working solution to samples at a v/v ratio of 1:50. Mix the cell suspension to disperse the fluorescent inhibitor reagent. If staining adherent cells, add fluorescent inhibitor reagent directly to the overlay media. The concentration of fluorescent inhibitor reagent should be optimized for each cell line, experimental condition, and method of analysis.

14.6 Incubate cells at 37°C protected from light, mixing gently every 10-20 mins to disperse the reagent. The incubation period may range from 30 mins to several hours and should be optimized for each cell line and experimental condition.

14.7 Wash cells. For trypsinized adherent (suspension) cells, go to Step 14.8 or for adherent cells, go to Step 14.9.

14.8 Wash trypsinized adherent (suspension) cells:

14.8.1 Add 2 mL 1X Cellular Wash Buffer and gently mix.

14.8.2 Centrifuge at 200 *x g* for 5-10 mins at RT.

14.8.3 Carefully aspirate supernatant.

14.8.4 Resuspend samples in 1-2 mL wash buffer and gently mix.

14.8.5 Centrifuge a second time at 200 *x g* for 5-10 mins at RT.

14.8.6 Carefully aspirate supernatant.

14.8.7 For flow cytometry analysis, two wash steps are generally sufficient. For microscopy or fluorescence plate reader analysis, repeat wash procedure a third time by following steps d-f.

14.8.9 Cells may be counter-stained with ancillary dyes like the nuclear stain Hoechst 33342 (go to Step 14.10) or other compatible dyes, or cells are ready for analysis (go to Step 14.11).

14.9 Wash adherent cells.

14.9.1 Carefully remove overlay media containing fluorescent inhibitor reagent and replace with 1X Cellular Wash Buffer.

14.9.2 Incubate 10 mins at 37°C to allow any unbound fluorescent inhibitor reagent to diffuse out of cells.

14.9.3 Carefully remove and replace wash buffer with fresh wash buffer.

14.9.4 Incubate 10 mins at 37°C.

14.9.5 Gently remove buffer and replace for a third wash step. Incubate 10 mins at 37°C.

14.9.6 Gently remove buffer. Cells may be counter-stained with ancillary dyes like the nuclear stain Hoechst 33342 (go to Step 14.10) or other compatible dyes, or cells are ready for analysis (go to Step 14.11).

Δ Note: To avoid losing cells that are no longer adherent during washing, spin down all overlay media and wash buffer and recombine washed cell pellets with the adherent samples prior to analysis.

14.10 To stain samples with Hoechst 33342 or other compatible fluorescent dyes:

14.10.1 Resuspend cells or replace overlay buffer.
14.10.2 Add Hoechst 33342 at 0.5% v/v, and incubate 10 to 20 mins at RT or 37°C. For example, if the cell suspension or overlay volume is at 1 mL, add 5 µL Hoechst 33342.
14.10.3 If using other compatible ancillary dyes, follow the manufacturer's instructions for staining samples.
Δ Note: Live/dead cell stains should not be used after trypsinization. Cell membranes exposed to trypsin could be transiently permeable to live/dead dyes for a variable time depending upon the cell line. To identify dead cells with a live/dead stain, perform the fluorescent inhibitor reagent and ancillary dye staining and wash steps prior to using trypsin. To avoid false positives, include another wash step to remove excess live/dead dye prior to trypsinization.

14.11 Read cells within 4 hrs or fix. Fixed cells may be stored protected from light on ice or at 2-8°C for up to 16 hrs.

- If analyzing with a fluorescence microscope, go to Section 15.
- If using a flow cytometer, go to Section 16.
- If using a fluorescence plate reader, go to Section 17.

15. Microscopy analysis

15.1 Resuspend cells or replace overlay media in 300-500 µL 1X Cellular Wash Buffer and place on ice. At this point, the cells may be stained with Propidium Iodide (PI) for bicolor analysis (Step 15.2), fixed for future viewing (Step 15.3), or observed immediately (Step 15.4).

15.2 To identify dead cells by staining with PI, add 2.5 µL PI to the 500 µL cell suspension. If different volumes were used, add it at 0.5% v/v. Incubate 5 mins at 37°C.

15.2.1 Wash cells to remove excess PI from the media. Centrifuge at 200 *x g* for 5-10 mins at RT.

15.2.2 Carefully remove and discard supernatants. Gently vortex pellets to disrupt clumping.

15.2.3 Resuspend cells in 300 µL 1X Cellular Wash Buffer and gently mix. Go to Step 15.3 or 15.4.

15.3 If not viewing immediately, cells may be fixed and viewed later. Fixed cells may be stored protected from light on ice or at 2-8°C for up to 16 hrs.

15.3.1 Add Fixative at a v/v ratio of 1:5-1:10.

15.3.2 Incubate 15 mins at RT in the dark.

15.3.3 Place cells onto a microscope slide and allow to dry.

15.3.4 Briefly wash cells with PBS.

15.3.5 Cover cells with mounting media and coverslip.

15.3.6 Store slides at 2-8°C for up to 16 hrs.

15.4 To view cells immediately, place 1 drop of cell suspension onto a microscope slide and cover with a coverslip.

15.5 Observe cells under a fluorescence microscope. Cells bearing active serine protease enzymes covalently coupled to Fam-fluorescent inhibitor reagent appear green, and those coupled to SR101-fluorescent inhibitor reagent appear red.

15.5.1 Observe cells labeled with FAM-fluorescent inhibitor reagent using a bandpass filter with excitation 490 nm and emission >520 nm to view green fluorescence.

15.5.2 View PI (red) under a broad bandpass filter with the excitation at 490 nm, emission >610; optimal settings would be 490 nm excitation and 617 emission; nuclei-bound PI has a maximum emission at 617 nm.

15.5.3 Observe cells labeled with SR101-fluorescent inhibitor reagent using a broad bandpass filter with excitation at 590 nm and emission >610 nm to view red fluorescence.

15.5.4 Hoechst 33342 (blue) can be seen using a UV-filter with excitation at 365 nm and emission at 480 nm.

16. Flow Cytometry analysis

16.1 Resuspend cells in 300 µL 1X Cellular Wash Buffer and place on ice.

16.2 Cells may be fixed for analysis up to 16 hrs later. Add Fixative at a v/v ratio of 1:5-1:10. Store samples at 2-8°C and protected from light.

16.3 Run the unstained controls. Generate an FSC vs SSC dot or density plot and gate on the population of interest. Adjust the voltages, if necessary, so that the cell population is easily distinguished.

16.4 For single-color analysis of FAM-fluorescent inhibitor reagent, a 488 nm blue argon laser or comparable can be used with the emission filter pairing that best approximates 530/30 (often FL-1/FITC channel). SR101-fluorescent inhibitor reagent is best analyzed using a laser that can efficiently excite the fluorophore, such as a yellow-green 561 nm laser. A green 532 nm laser with a 610/20 filter pairing has also been shown to be effective. If yellow-green or green laser options are unavailable, a standard 488 nm blue excitation laser paired with an appropriate emission filter set, such as 585/40 (often FL-2/PE channel), can be used.

16.5 Run single color controls. Generate a histogram with the log FL channel on the x-axis versus the number of cells on the y-axis. Cells with lower levels of serine protease activity will fall within the lower log fluorescence output decades on the x-axis and the cells with increased levels of serine protease activity will appear as a shoulder or as a separate peak on the right side of the negative peak histogram (Figure 1). Adjust the voltage on the fluorescence channel, if necessary, to ensure fluorescence is on scale and serine protease positive and negative populations are distinguished.

16.6 For dual-color analyses, run each single-color control. Adjust compensation to remove spectral overlap from interfering FL channels.

16.7 Run experimental samples and analyze.

17. Fluorescence plate reader analysis

17.1 Determine the concentration and compare the cell density of each sample. The non-induced population may have more cells than the induced population (if the induction process caused any cell death), as some cells in the induced samples may be lost during the wash steps. Adjust the volume of the cell suspensions to equalize the cell density. When ready to read, cells should be >3 x 10^6 cells/mL. Adherent cells should be cultured to ~90% confluency.

17.2 If using suspension cells, pipette 100 µL stained and washed cells per well into a black microtiter plate. Do not use clear plates. If using a bottom-reading instrument, use a plate with black walls and a clear bottom. Analyze at least 2 aliquots per sample. Avoid bubbles.

17.3 Perform an endpoint read:

17.3.1 For FAM-fluorescent inhibitor reagent: Set the excitation wavelength at 488 nm and the emission wavelength at 530 nm; if possible, use a 515 nm cut-off filter. FAM-fluorescent inhibitor reagent excites at 488-492 nm and the emits at 515-535 nm.

17.3.2 For SR101-fluorescent inhibitor reagent: Set the excitation wavelength at 590 nm and the emission wavelength at 620 nm; if possible, use a 610 nm cut-off filter. SR101-fluorescent inhibitor reagent excites at 586 nm and emits at 605 nm.

18. Notes

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