FlowCellect™ LC3-GFP Reporter Autophagy Assay Kit

100 Tests

Cat. No. FCCH100170 and FCCH100181

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Not for use in diagnostic procedures.

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Application

Autophagy is an intracellular catabolic pathway that causes cellular protein and organelle turnover, and it is associated in diverse diseases including Alzheimer's disease, aging, cancers, and Crohn's disease. It is a tightly regulated process that plays a normal part in cell growth, development, and cellular homeostasis. Autophagy functions as a housekeeping mechanism by disposing of aging and/or dysfunctional proteins and organelles by sequestering and priming for lysosomal degradation. Increasing evidence suggests that not just apoptosis, but autophagy can contribute to cell death and greatly influence general cell health. Malfunctions of autophagy can influence longevity and productivity of cells to function at full capacity.

During autophagy, LC3 protein is translocated from the cytoplasm to the autophagosome where it is targeted to the lysosome for degradation. The process of autophagy can be categorized into four distinct stages (See figure 1):

1) *Induction and LC3 Translocation:* The process is initiated by external/internal stimuli (e.g. nutrient depletion);
2) *Autophagosome formation*: Unwanted cytosolic proteins and aging organelles are sequestered by a double membrane vesicle, i.e.—"autophagosome". Formation of this vesicle is coordinated by complexes of Atg proteins (Atg5 and Atg12) that are conjugated, enabling the recruitment of LC3;
3) *Lysosomal docking and fusion:* LC3 protein regulates traffic between autophagosome to lysosome. (LC3-I is cytoplasmic; LC3-II is lipidated and sequestered into autophagosomal membrane);
4) *Degradation:* Fusion with the lysosome and subsequent breakdown of the autophagic vesicle and its contents.

Millipore's FlowCellect™ LC3-GFP Reporter Autophagy Assay Kits provide a quantitative solution for studying autophagy and measuring potency of autophagy inducers using flow cytometry. This kit has three unique features: A) the use of selective permeabilization solution discriminates between cytosolic LC3 from autophagic LC3 by extracting the soluble cytosolic proteins, while protecting LC3 which has been sequestered into the autophagosome; B) the use of monomeric GFP is used as a reporter to facilitate the translocation of the fusion protein, as other forms of GFP form dimers and aggregate when over-expressed in the cells, thus making it difficult to be extracted from cytoplasm and impossible to measure translocation by flow cytometry; and C) since autophagy is a constitutive cellular degradation process, the use of an autophagy detection reagent (Autophagy Reagent A) will prevent the lysosomal degradation of LC3, allowing its quantification by flow cytometry. Two host cell lines are offered in either CHO (Chinese Hamster Ovary) or U20S (human osteosarcoma) to assist in your autophagy research. The CHO reporter cell line is ideal for flow cytometry applications, while U20S is suitable for both imaging and flow cytometry.

By having the ability to measure and quantify autophagy, we are able to screen and rank order autophagy inducers or inhibitors, monitor cell culture health and protein turnover rate, study the mechanisms of protein degradation, and identity new autophagy targets and pathways leading to aging and neurodegenerative diseases.

The LC3-GFP reporter cell lines and autophagy enabling reagents are optimized on guava® bench top flow cytometers. These kits can be used on any flow cytometer or imaging device following the same protocol, which will provide researchers a reliable, quantitative, and fully validated solution to study autophagy. The LC3-GFP reporter cell line provided in the kits has been carefully chosen to

ensure maximal performance alleviating the need for any additional optimization. These kits contain optimized autophagy enabling reagents to provide researchers with a complete solution for autophagy analysis.

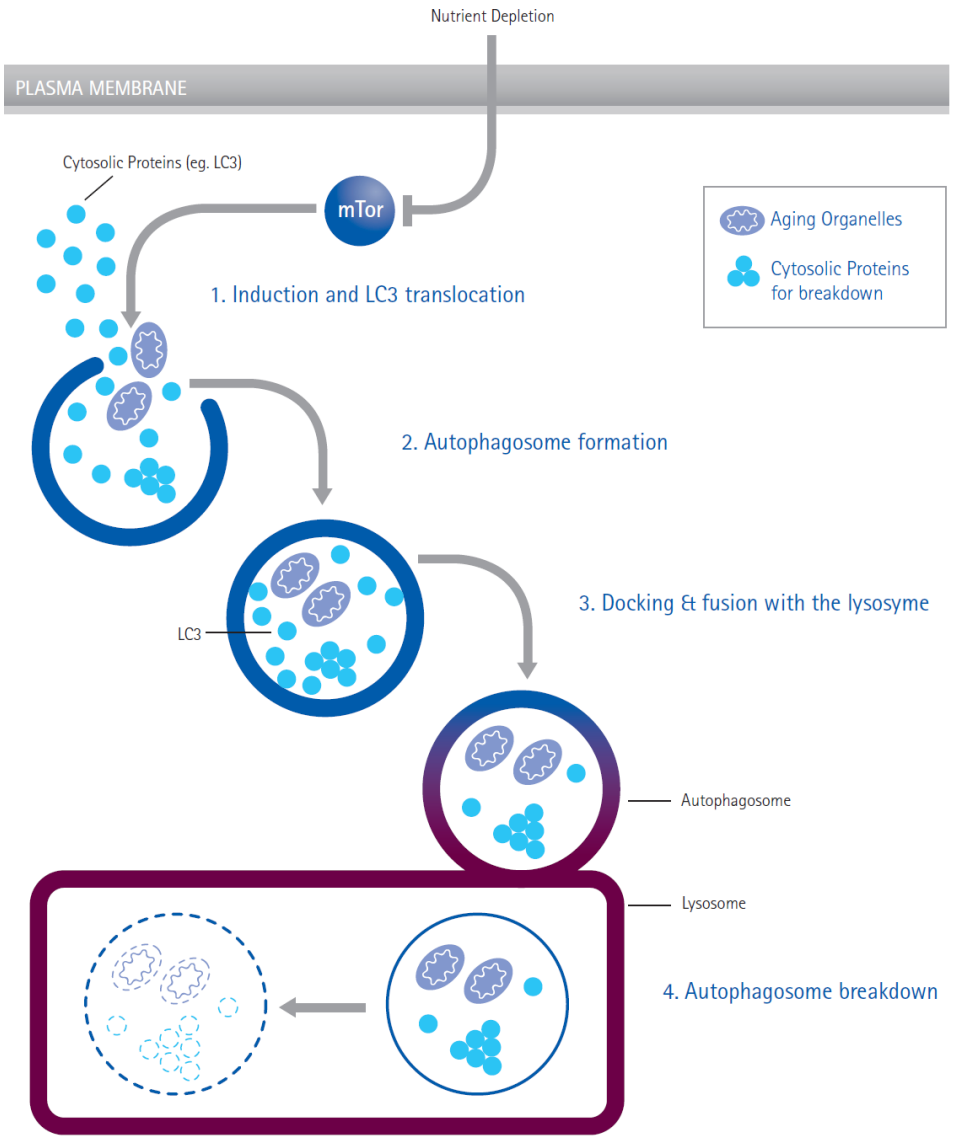

Figure 1. Autophagy: Four Stages of Autophagy Autophagy can be induced by nutrient depletion or inhibition of mTOR pathway. During autophagy, cytosolic proteins and aging organelles are sequestered by a double membrane vesicle to form autophagosomes. One of the hallmarks of autophagy is translocation of LC3 from the cytoplasm to the autophagosome. Autophagosome then fuses with the lysosome to cause the breakdown of autophagosome vesicle and its contents, including LC3. This process can be visualized using either an LC3-GFP fusion protein or and anti-LC3 antibody.

Test Principle

Discrimination between cytosolic and autophagosome associated LC3 is achieved by monitoring the translocation of LC3-GFP fusion protein using flow cytometry. This kit provides the reagent for the disruption of the cell plasma membrane using a proprietary selective permeabilization solution (figure 2). The selective permeabilization solution will extract cytosolic LC3-GFP by flushing away during washing steps. LC3-GFP translocated into the autophagosome is protected from the extraction and remains intact inside autophagosome, thereby allowing its fluorescence to be measured by flow cytometry or imaging. Another attractive feature is the LC3-GFP reporter cell lines provided in the kits contain a monomeric GFP instead of the traditional GFP which exists as a dimer. The monomeric nature is intended to minimize undesirable multimerization and aggregation of the fusion proteins. Forced dimerization as seen with normal GFP resulting in aggregation will not be optimal for measurements of translocation events by flow cytometry or other applications. Since autophagy is a constitutive cellular degradation process, the use of an autophagy detection reagent (Autophagy Reagent A) will prevent the lysosomal degradation of LC3-GFP, allowing for quantification of its fluorescence.

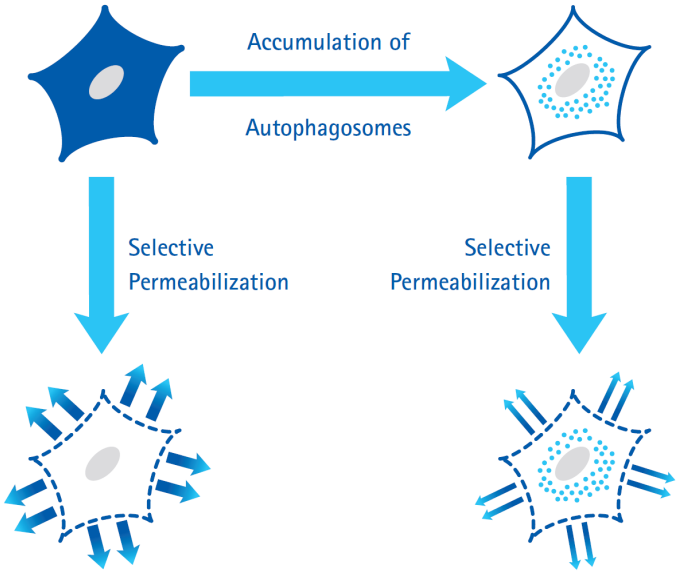

Figure 2. Selective Permeabilization helps discriminate cytosolic from autophagic LC3 Discrimination between GFP-cytosolic LC3-I from autophagosome associated GFP-LC3-II is achieved by disruption of cell PM by using an autophagy enabling solution (Autophagy Reagent B). This selective permeabilization will "release" cytosolic LC3 by flushing away during washing steps. LC3-II trapped in the autophagosome remains intact and fluorescence can be measured.

In various case studies, we have validated the useful application of a LC3-GFP reporter by screening well known autophagy inducers and inhibitors, rapamycin and dynasore, respectively. And in addition to hit identification, we have further characterized the activity of other various small molecules such as STF-6227 and PI-103 which are well known autophagy inducers by performing dose response curves to derive EC_{50} values.

Millipore's LC3-GFP Reporter Autophagy Assay Kits include one immortalized LC3-GFP reporter cell line to measure and track the levels of LC3 within the cell. The autophagy detection reagents and cell line have been optimized together to ensure the ability to measure and discriminate between cytosolic and lipidated LC3 to accurately measure the autophagic process. Sufficient enabling reagents are provided to perform 100 tests. Detailed assay instructions are included to assist in analysis.

Case Study #1(optional): Assessment of autophagic activity by autophagosomes using both an autophagy inducer (Rapamycin) and inhibitor (Dynasore)

A case study was conducted to evaluate the effects of both Rapamycin and Dynasore treatments on autophagy.

Rapamycin is an mTOR inhibitor and has been indicated to induce autophagy. mTOR is a member of the PI3-kinase family and is a central modulator of cell growth in response to environmental signals. It plays a critical role in transducing proliferative signals by activating downstream protein kinases that are required for both ribosomal biosynthesis and translations. 2000 Nobel Laureate Paul Greengard has demonstrated that a small molecule enhancer of Rapamycin - SMER28, decreases levels of amyloid-β (Ab) peptide, which is a hallmark of Alzheimer's disease. Autophagy is one major cellular pathway leading to the removal of such proteins. By targeting mTOR, rapamycin mimics the cellular starvation response by inhibiting signals required for cell cycle progression, cell growth, and proliferation and leads to the activation of autophagy (figure 5).

Dynasore is a cell-permeable inhibitor of dynamin which has been indicated to inhibit autophagy. Dynamin is essential for clathrin-dependent coated vesicle formation. Dynamin is required for membrane budding at a late stage during the transition from a fully formed pit to a pinched off vesicle. Dynamin may also fulfill other roles during earlier stages of vesicle formation. Dynasore acts as a potent inhibitor of endocytic pathways known to depend on dynamin by rapidly blocking coated vesicle formation within seconds of dynasore addition. As a result, Dynasore will inhibit autophagosome formation, which in effect, will inhibit autophagy (figure 6).

Case Study #2 (optional): Deep dive small molecule evaluation by performing dose response curves for EC_{50} determination by flow cytometry

Another case study was conducted to perform a deep dive analysis of small molecule autophagy inducers, STF-6227 and PI-103. STF-6227 and PI-103 have been indicated as autophagy inducers, and by using our selective permeabilization method along with the LC3-GFP reporter cell line we were able to utilize this assay as a viable screening tool. Since structure-activity relationships (SAR) of small molecules are critical in identifying selective autophagy inducers; the level of LC3-II was determined by flow cytometry as indicated by the mean fluorescence intensity of the signal relative to the baseline negative control. In figure 7, STF-6277 and PI-103 were incubated for 8 hours in a 12 point, half-log dose dependent manner. From these values, a dose response curve is developed and EC_{50} values determined. By implementing this method autophagy compounds can be rank ordered to help complement any SAR campaigns during drug development. This data clearly illustrates the wide dynamic range of the reporter cell line as well validates the effective use of the autophagy enabling solutions.

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Kit Components

- LC3-GFP reporter cell line (stored in -80℃ for sh ort term; in liquid nitrogen for long term):
    - o CHO cell background for FCCH100170 (Part No. CF200095) One vial containing 5 million cells.

      or,
    - o U20S cell background for FCCH100181 (Part No. CF200096) One vial containing 5 million cells

CF200097: Autophagy Reagent Pack (stored at 2℃ - 8 ℃) :

- Autophagy reagent A: (Part No. CS208212) One vial (lyophilized)
- Autophagy reagent B: (Part No. CS208215) One vial containing 1 mL
- 5X Assay Buffer: (Part No. CS202124) One bottle containing 55 mL

Materials Not Supplied

1. Test tubes for sample preparation and storage
2. Tissue culture reagents, i.e. HBSS, PBS w/o Ca^{2+} or Mg^{2+}, cell dislodging buffers, etc.
3. Pipettors with corresponding tips capable of accurately measuring 10 – 1000 µL
4. Tabletop centrifuge capable of achieving 300 x g
5. Mechanical vortex
6. Flow Cytometer
7. Deionized water (for reagent dilutions)
8. Rapamycin reagent (EMD Chemicals; Part No. 553210)
9. Dynamin Inhibitor I, Dynasore reagent (EMD Chemicals; Part No. 324410)
10. STF-62247, Autophagy Inducer (EMD Chemicals; Part No. 189497)
11. PI-103, ATP-competitive inhibitor of PI3-K and mTOR (EMD Chemicals; Part No. 528100)

Precautions

- The instructions provided have been designed to optimize the kit's performance. Deviation from the kit's instructions may result in suboptimal performance and may produce inaccurate data.
- Some assay components included in the kit may be harmful. Please refer to the MSDS sheet for specific information on hazardous materials (MSDS forms can be found on the web page or by contacting Millipore technical services).
- During storage and shipment, the autophagy enabling reagents may condense within the vial. For maximum recovery of the product, centrifuge original vial prior to removing cap.
- Cell line must be stored either in -80℃ (short ter m) or liquid nitrogen (long term) upon receipt. Keeping cells warmer than -20℃ can compromise the integrity of the product.
- Do not use reagents beyond the expiration date of the kit.

Storage

This is a dual storage kit. All reagents must be stored at 2 - 8ºC, and the cell line must be stored at either -80℃ (short term) or in liquid nitrogen (lo ng term).

All kit components are stable up to six (6) months from date of receipt if stored and handled correctly. **Please avoid repeated changes in temperature as this will affect the integrity of the product.**

Preparation of Reagents

1. **Autophagy Reagent A**

   This material is supplied in a lyophilized vial. Prior to use, reconstitute the contents of the vial in 250 µL deionized water.

   Note: It is recommended to aliquot multiple vials and keep stored at -20℃. Avoid repeated freeze and thaw.

2. **Autophagy Reagent B**

   Autophagy reagent B is supplied at 10X concentration and should be diluted to 1X with deionized water prior to use. Prepared 1X Autophagy Reagent B is stable up to one year. Store at 2 - 8ºC.

3. **Assay Buffer**

   Assay Buffer is supplied at 5X concentration and should be diluted to 1X with deionized water prior to use. Prepared 1X Assay Buffer is stable up to one year. Store at 2 - 8ºC.

Assay Instructions

Storage and Handling / Cell Propagation and Maintenance

Immediately upon receipt, thaw cells or place cells in-80℃ (short term storage) or in liquid nitrogen (long term storage)

1. Thaw cells rapidly by removing from liquid nitrogen by immediately immersing in a 37ºC water bath. Immediately after thaw, sterilize the exterior of the vial with 70% ethanol. Transfer contents of the vial to a T-75 flask containing 20-25 mL growth media. Place the flask in a humidified incubator at 37ºC with 5% CO_2.
2. After 8-24 hours, all live cells will be attached. Viability of the cells is expected to be 60-90%. At this time, replace growth media to remove any residual DMSO and return to incubator.
3. When cells are approximately 80% confluent, passage the cells as follows: Remove media and wash once with HBSS without Ca^{++} and Mg^{++} (10 mL/T-75). Add 1 mL of a mild enzyme to dissociate adherent cells from the flask (Accutase; Cat. No. SCR005 from EMD Millipore or 0.05% trypsin/0.2 g/L EDTA; Cat No. SM-2002-C). Place in humidified incubator at 37ºC with 5% CO_2 until cells begin to round up and detach (5-10 minutes). Gently tap the side of the flask to dislodge the cells. Neutralize cell dissociating enzyme by addition of 4 mL growth media per 1 mL cell dissociating enzyme.

4. Cell passage is typically at 1:30 every 3-4 days. Passage ratios may be varied according to requirements of the investigator.

Cell Culture Media

CF200095 (CHO): Growth Media

- F12-K containing 2 mM L-glutamine
- 10% heat-inactivated FBS (EMD Millipore, Cat. No. ES-009-B) NOTE: Must heat-inactive before use.
- 1X Pen-Strep (from 100X stock; EMD Millipore, Cat. No. TMS-AB2-C)
- 250 μg/mL Genetecin/G-418

CF200095 (CHO): Plating Media

- F12-K containing 2 mM L-glutamine
- 10% heat-inactivated FBS
- 1X Pen-Strep

CF200096 (U20S): Growth Media

- DMEM with 4.5 g/L glucose and 4 mM glutamine (EMD Millipore, Cat. No. SLM-020-A)
- 10% heat-inactivated FBS (EMD Millipore, Cat. No. ES-009-B) NOTE: *Must heat-inactive before use.*
- 1X Non-essential amino acids (from 100X stock; EMD Millipore, Cat. No. TMS-001-C)
- 10mM HEPES (from 1 M HEPES, EMD Millipore, Cat. No. TMS-003-C)
- 1X Pen-Strep (from 100X stock; EMD Millipore, Cat. No. TMS-AB2-C)
- 250 μg/mL Genetecin/G-418

CF200096 (U20S): Plating Media

- DMEM with 4.5 g/L glucose and 4 mM glutamine
- 10% heat-inactivated FBS
- 1X Non-essential amino acids
- 10mM HEPES
- 1X Pen-Strep

I. General Assay Protocol (To monitor autophagosomes)

1. Seed approximately 3 to 4 million cells (CHO) or 4 to 5 million cells (U20S) into each of two T-75 flasks overnight in a humidified 37℃ incubator with 5% CO_2. Cells should be at about 80% confluent the next day.

2. The next day, aspirate and wash cells in both T-75 flasks with 10 mL 1X HBSS. To one flask, label as "untreated" and add 20 mL Earle's Balanced Salt Solution (EBSS). To the other flask, label as "treated" and add 20 mL of EBSS + 100µM of Autophagy Reagent A. Incubate both flasks in a humidified incubator at 37ºC with 5% CO_2 for 2 hours.

3. Aspirate media and wash both flasks with 10mL of 1x HBSS. Detach and transfer cells to labeled conical tubes.

4. Determine cell numbers by using ViaCount or a hemacytometer and note cell viability. Healthy cells should be above 90% viable.

5. Spin down cells at 300 x g for 5 minutes at room temperature and aspirate media.

6. Resuspend each sample to 2 million cells per mL in 1X Assay Buffer.

7. Add 50 µL of cell suspension into the well of a "V" bottomed 96-well plate (100K cells/well) if using a guava HT instrument. NOTE: See manual for instrument compatible plates. If not using a guava HT instrument, add 100 µL into sample tubes.

8. Wash cells by adding 150 µL of a 1X Assay Buffer into each well and spin at 300 x g for 5 minutes.

9. Add 100 µL of 1X Autophagy Reagent B to each well, followed by gentle resuspension to ensure proper distribution, and immediately spin at 300 x g for 5 minutes.

10. Wash once with 1X Assay Buffer to remove residual 1X Autophagy Reagent B and spin at 300 x g for 5 minutes.

11. Resuspend each well in 200 µL of 1X Assay Buffer (or 500 µL of 1X Assay Buffer in sample tubes) and acquire data.

II. General Protocol for Compound Screening (optional)

a. Compound Hit Identification:

1. Seed 30K cells into a 96-well plate overnight in a humidified 37℃ incubator with 5% CO_2. Cells should be at about 80-90% confluent the next day.

2. Dilute each compound to a final concentration 10 μM in plating media or media prefer by the investigator (NOTE: Starting sample concentration must be determined by the researcher's own discretion).

3. Remove plating media from each well and discard.

4. Add 200 μL of the diluted compounds to the appropriate wells (or sample tubes) and incubate to the desired time point (e.g. 1 to 8 hour incubation; to be determined by the researcher).

5. Thirty minutes before the end of the scheduled time point, dilute Autophagy Reagent A to a final of 1 mM in plating media.

6. Add 20 μL of the 1 mM Autophagy Reagent A to each well (final of 100 μM).

7. Incubate the cells for 1 hour at 37℃.

8. Aspirate plating media and wash cells once with 200 μL of 1X HBSS.

9. Add 100 μL of a mild enzyme (e.g. Accutase) to each well.

10. Allow cells to incubate at 37℃ for 5 minutes to detach cells.

11. During the incubation step, add 100 μL of plating media to a guava compatible "V"-bottomed 96-well plate.

12. After cell incubation, gently resuspend cells in the 96-well plate to dislodge cells, followed by transferring cells to the "V"-bottomed 96-well plate for a total of 200 μL in each well.

13. Spin at 300 x g for 5 minutes at room temperature and discard supernatant.

14. Add 100 μL of 1X Autophagy Reagent B to each well and immediately spin at 300 x g for 5 minutes. Carefully discard supernatant from each well.

15. Wash once with 1X Assay Buffer to remove residual 1X Autophagy Reagent B and spin at 300 x g for 5 minutes. Carefully discard supernatant from each well.

16. Resuspend cells in each well with 200 μL 1X Assay Buffer.

17. Acquire samples.

b. Compound Dose Response Curves

1. Seed 30K cells into a 96-well plate overnight in a humidified 37℃ incubator with 5% CO_2. Cells should be at about 80-90% confluent the next day.

2. Dilute each compound to a final concentration 10 μM in plating media or media prefer by the investigator (NOTE: Starting sample concentration must be determined by the researcher's own discretion).

3. Serial dilute each compound of interest in preparation for cell treatment and determination of dose response curves.

4. Aspirate media from each well and discard (Optional: Wash once with 1X HBSS and aspirate to remove any residual growth media from cells).

5. Add 200 μL of the diluted compounds to the appropriate wells and incubate to the desired time point.

6. Thirty minutes before the end of the scheduled time point, dilute Autophagy Reagent A to a final of 1 mM in plating media.

7. When the desired time point is up, add 20 μL of the 1mM Autophagy Reagent A to each well (for a final concentration of 100 μM).

8. Incubate the cells for 1 hour at 37℃.

9. Aspirate plating media and wash cells once with 200 μL of 1X HBSS.

10. Add 100 μL of a mild enzyme (e.g. Accutase) to each well.

11. Allow cells to incubate at 37℃ for 5 minutes t o detach cells.

12. During the incubation step, add 100 μL of plating media to a guava compatible "V"-bottomed 96-well plate.

13. After cell incubation, gently resuspend cells in the 96-well plate to dislodge cells, followed by transferring cells to the "V"-bottomed 96-well plate for a total of 200 μL in each well.

14. Spin at 300 x g for 5 minutes at room temperature and discard supernatant.

15. Add 100 μL of 1X Autophagy Reagent B to each well and immediately spin at 300 x g for 5 minutes. Carefully discard supernatant from each well.

16. Wash once with 1X Assay Buffer to remove residual 1X Autophagy Reagent B and spin at 300 x g for 5 minutes. Carefully discard supernatant from each well.

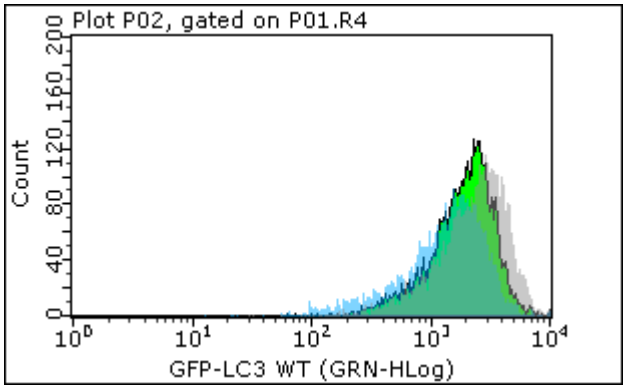
17. Resuspend cells in each well with 200 μL 1X Assay Buffer.

18. Acquire samples.

Sample Data

Reporter Assay: Flow cytometry detection of LC3 translocation via autophagosomes by addition of a lysosome inhibitor (using LC3-GFP reporter)

A. Without Selective Permeabilization



B. With Selective Permeabilization

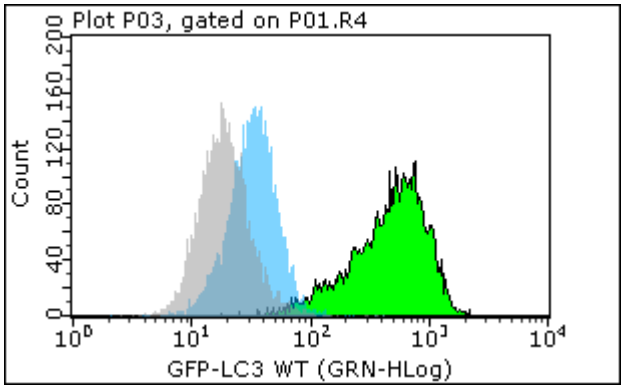
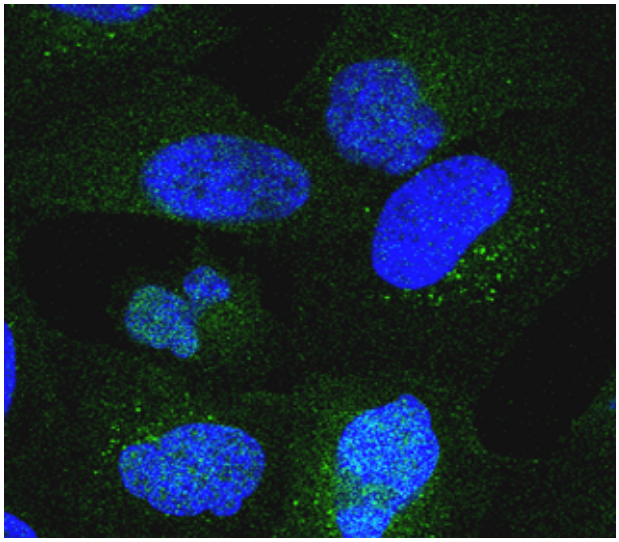


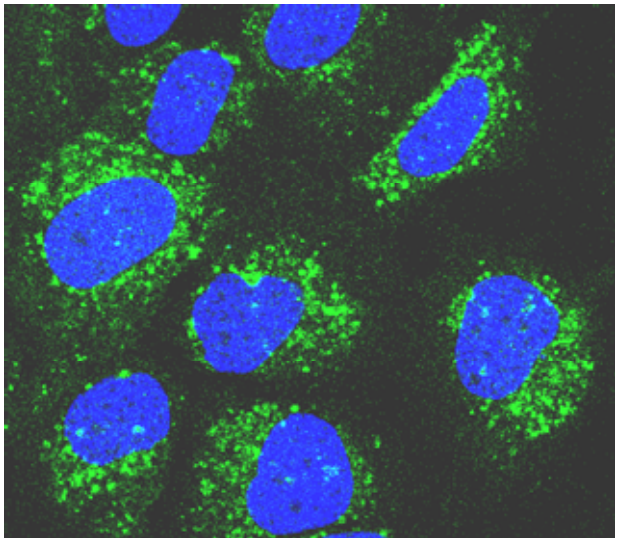
Figure 3. LC3-GFP stable reporter cell line for detecting the rate of autophagy and for drug screening
In (A), without Selective Permeabilization no shift of LC3-GFP level is detected using flow cytometry before and after starvation (induction of autophagy). The position of the histograms indicates the high level of LC3-GFP expression in the cytoplasm.
In (B), with Selective Permeabilization LC3-GFP level remains high in autophagosomes when starved in the presence of lysosome inhibitor (green); even without the inhibitor, a slight shift is observed when starved (blue). All the cytosolic LC3-GFP is washed away if no autophagy is induced by starvation (gray).

Legend:
Non-starved (control for no autophagy)
Starved in the absence of lysosome inhibitor
Starved in the presence of lysosome inhibitor

A. Control (Uninduced cells)

B. Induced Autophagy

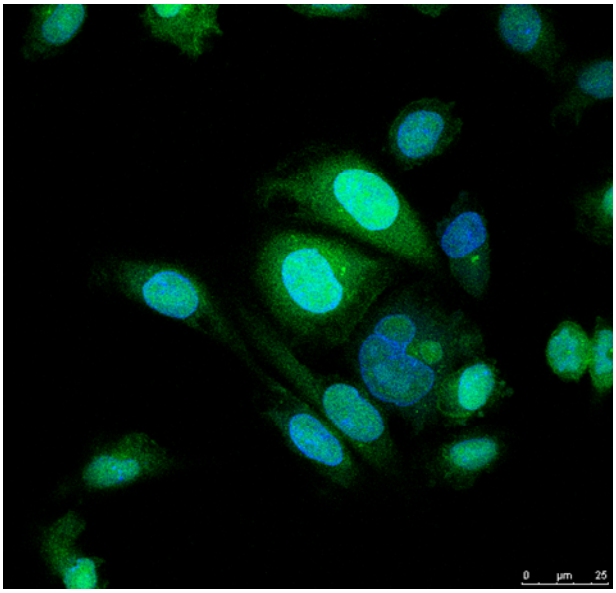


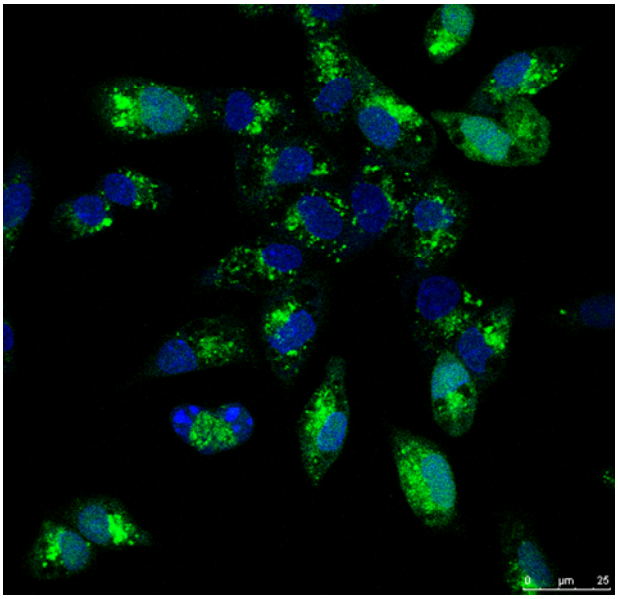


U20S cells

A. Control (Uninduced cells)

B. Induced Autophagy





CHO cells

Figure 4. Image analysis of LC3-GFP reporter cell line Cells are nutrient deprived to induce autophagy and then treated with autophagy reagent A to prolong the signal for detection of translocated LC3-GFP in the autophagosomes, which is a hallmark of the autophagic process (B). As illustrated, LC3-GFP puncta is visualized as green dots. Control cells which are uninduced are shown in (A). Cell nuclei stained with Dapi (blue).

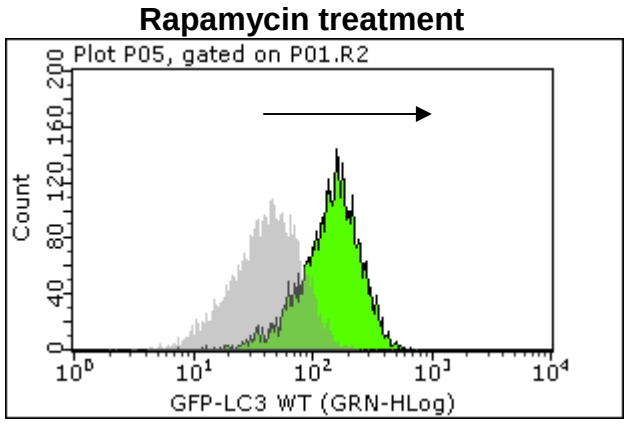


Figure 5. Rapamycin induces Autophagy through the mTOR pathway Rapamycin is an inhibitor of the mTOR pathway, and by targeting mTOR, rapamycin mimics the cellular starvation response and leads to activation of autophagy as illustrated by the right shift of the histogram (green). Cells were treated with 400 nM Rapamycin for 48 hours prior to data acquisition.

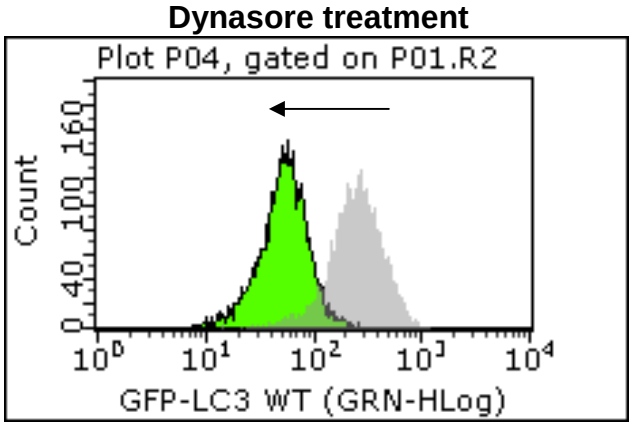


Figure 6. Dynasore inhibits Autophagy by inhibition of autophagosome formation Dynasore is a cell-permeable inhibitor of dynamin. Dynamin is essential for clathrin-dependent coated vesicle formation. Dynasore acts as a potent inhibitor of endocytic pathways known to depend on dynamin by rapidly blocking coated vesicle formation within seconds of dynasore addition. As a result, Dynasore will inhibit autophagosome formation, which in effect, will inhibit autophagy as illustrated by the left shift of the histogram (green). Cells were treated with 80 μM Dynasore for 3 hours prior to data acquisition.

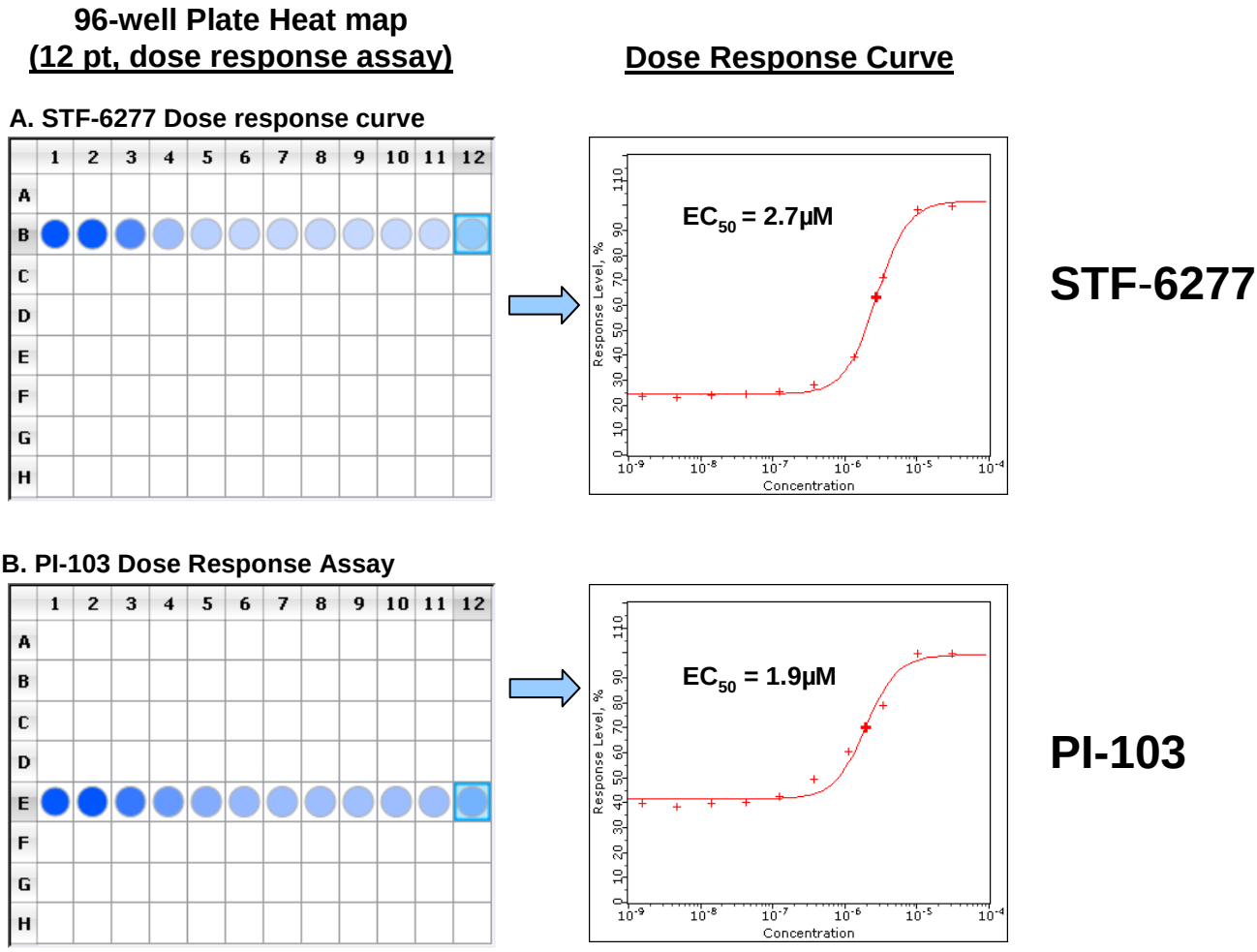


Figure 7. Deep dive small molecule evaluation by performing dose response curves for EC50 determination by flow cytometry using the InCyte™ Software Module STF-6227 and PI-103 have been indicated as autophagy inducers, and by using our selective permeabilization method along with the LC3-GFP reporter cell line we were able to utilize this assay as a viable screening tool. STF-6277 (A) and PI-103 (B) were incubated for 8 hours in a 12 point, half-log dose dependent manner.
By implementing this method autophagy compounds can be rank ordered to help complement any SAR campaigns during drug development. This data clearly illustrates the wide dynamic range of the reporter cell line as well validates the effective use of the autophagy enabling solutions.

Technical Hints

- If minor precipitate is detected in the 10X Wash Buffer place the bottle in a warm water bath for 15 minutes, followed by mixing the contents on a mechanical vortex.
- Make sure that the cell line is greater than 80% viability prior to assay and is maintained under drug selection media to ensure ideal expression LC3-GFP over time.
- For drug treatments, all incubation times and sample concentrations must be optimized at the researchers own discretion. Some guidelines for drug treatment are provided in this kit but can be modified to suit the researcher's experimental needs.
- Do not mix or interchange reagents from various kit lots.

Troubleshooting

Assay Step	Potential Problem	Experimental Suggestions
Reagent Preparation	Precipitation found in 10X Wash buffer	• If storing at -20ºC, precipitate can form in the 10X wash buffer. Prior to use, place bottle in a 37ºC water bath, swirling the contents occasionally. If this does not remove the precipitate completely, allow 10X Wash Buffer to sit at room temperature overnight.
Acquisition	Acquisition rate decreases dramatically	This usually indicates that the fluid pathway on the instrument may be blocked. This can be alleviated by the following: • Decreasing number of cells for analysis. Guava flow cytometers have the capacity of analyzing a steady stream of 300 – 500 cells per microliter. Any cell densities in excess can essentially block the normal flow, causing disruption during the assay. Decrease the number of cells being analyzed by diluting the sample to approximately 0.5 million cells per milliliter. • Adherent cells can result in cellular clumping. Use a more aggressive enzyme for dissociation such as trypsin during cell harvesting should help keep cells in single suspension. Alternatively, using a cell strainer can help disrupt cell clumping if needed. • After many uses, it is possible that the fluid system on any standard flow cytometer will require cleaning. Run standard cleaning procedures to clean the fluid system during or after an assay. This will prevent any material from forming where the steady flow steam takes place.
Cellular Analysis	A loss or lack of signal	• Cell numbers may need to increase. Cell loss is common during washing steps in the assay procedure. A substantial decrease in cell numbers can lead to a loss of signal. Make sure that cell density remains at approximately 0.5 million cells per milliliter during analysis.
Cellular Analysis	Variability in day to day experiments	• Monitor experimental cell cultures to ensure that cell viability and cell numbers being analyzed are consistent. Any drop in cell numbers or viability can influence experimental results. • When using any guava easyCyte™ instrument for flow analysis, make sure that a quality check on the instrument (e.g. calibration) is performed on a daily basis prior to use. (**See Analytical Sensitivity and Detection Limits Section for Guava Check standards*)

For Technical Service, please visit www.millipore.com/techservice.

References

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