



# **FlowCollect™ Autophagy LC3 Antibody-based Assay Kit (100 tests)**

Catalog No. FCCH100171

**FOR RESEARCH USE ONLY**  
Not for use in diagnostic procedures.

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## Introduction

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 through sequestering and priming proteins for lysosomal degradation. Increasing evidence suggests that not just apoptosis, but also autophagy, can contribute to cell death and greatly influence general cell health. Malfunctions of the autophagy process are proposed to influence cell health longevity and the capability 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):

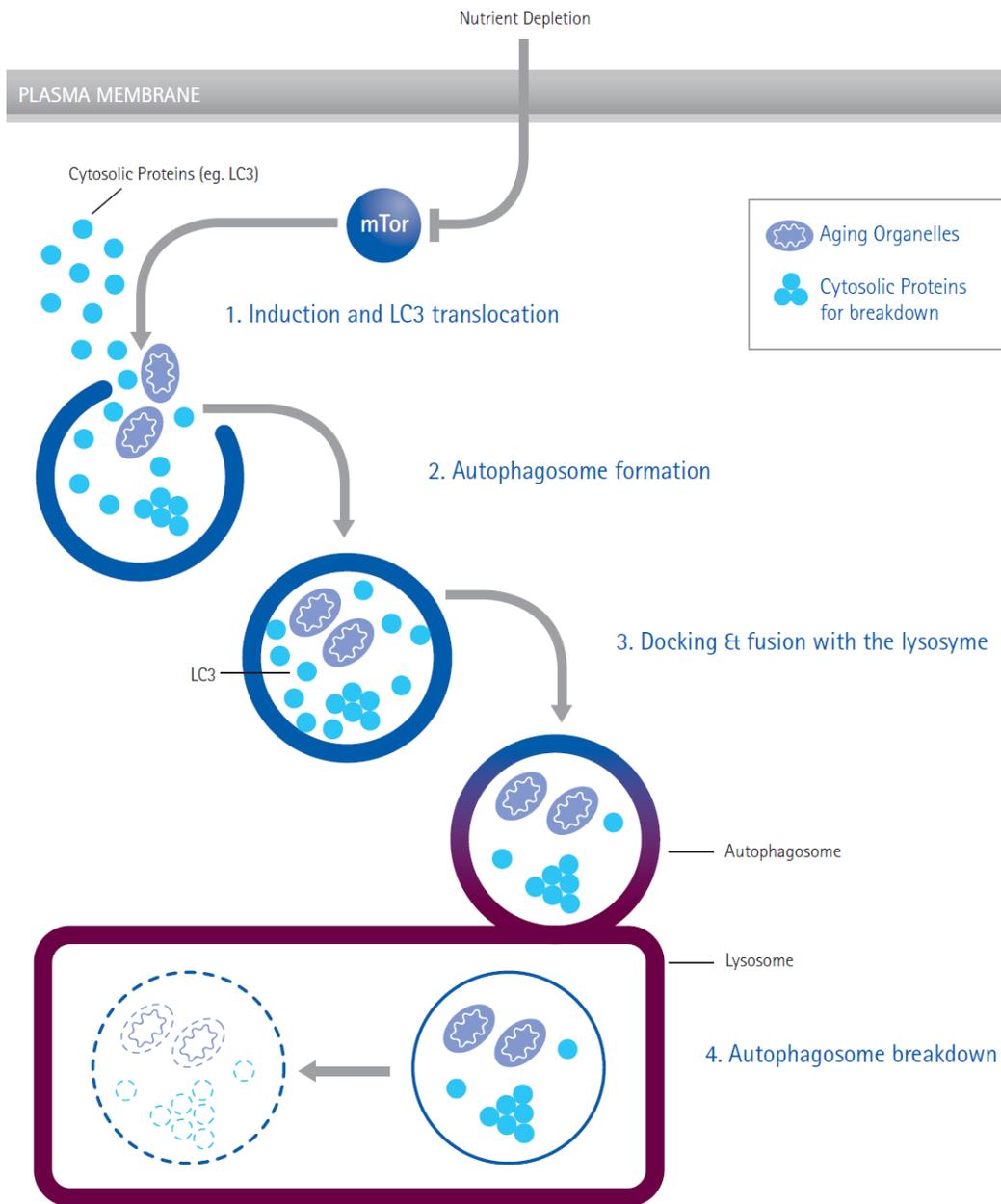
- 1) Induction and LC3 Translocation: The process is initiated by external/internal stimuli (e.g. nutrient depletion);
- 2) Autophagosome formation: Unwanted cytosolic proteins and aging organelles are sequestered by a double membrane vesicle, i.e.—"autophagosome". Formation of this vesicle is coordinated 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 FlowCollect™ Autophagy LC3 Antibody-based Assay Kit provides a quantitative solution for the study of autophagy and potency evaluation of autophagy inducers using flow cytometry. This kit contains two key detection reagents to help facilitate the monitoring of lipidated LC3-II in a given cell system:

- ❖ 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;
- ❖ 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;

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 identify new autophagy targets and pathways leading to aging and neurodegenerative diseases.

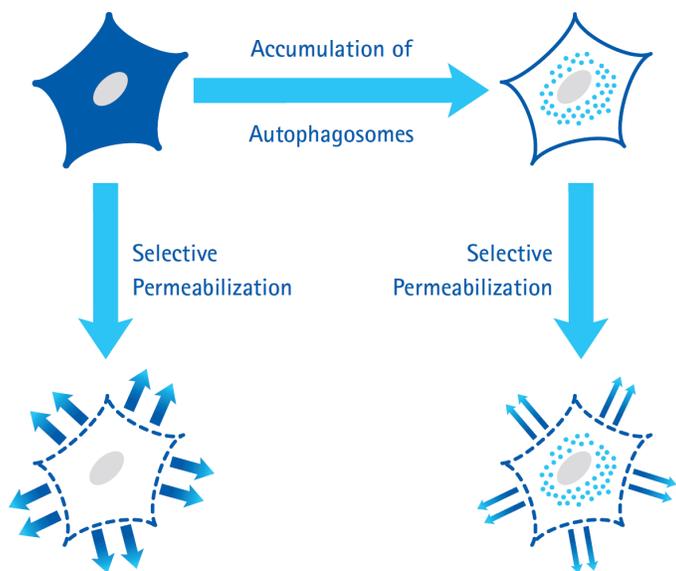
The anti-LC3 FITC conjugate and autophagy enabling reagents are optimized on guava® bench top flow cytometers and provide a complete solution for autophagy analysis. This kit can be used on any flow cytometer or imaging device by following the same protocol, offering researchers a reliable, quantitative, and fully validated solution to study autophagy. The anti-LC3 antibody provided in the kit has been carefully evaluated to ensure optimal performance, alleviating the need for any additional validation of the kit reagents.



**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 a fully validated anti-LC3 antibody.

## Test Principle

Discrimination between cytosolic and autophagosome associated LC3 is achieved by monitoring the translocation of LC3 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 by flushing away during washing steps. LC3 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. 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 for quantification of its fluorescence.



**Figure 2. Selective Permeabilization helps discriminate cytosolic from autophagic LC3** Discrimination between cytosolic LC3-I from autophagosome associated 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 the anti-LC3/FITC antibody conjugate 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 PiKyve and PI-103 which are well known autophagy compounds by performing dose response curves to derive EC<sub>50</sub> values.

Millipore's FlowCelect™ Autophagy LC3 Antibody-based Assay Kit include an anti-LC3 mouse monoclonal antibody conjugated to FITC, used to measure and track the levels of LC3 within the cell. The autophagy detection reagents and antibody 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- $\beta$  (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 4).

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 5).

### ***Case Study #2 (optional): Deep dive small molecule evaluation by performing dose response curves by flow cytometry***

Another case study was conducted to perform comprehensive analysis of small molecule autophagy compounds, PIKfyve and PI-103. PI-103 has been indicated as an autophagy inducer, and by using our selective permeabilization method along with the anti-LC3/FITC conjugated antibody 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 6, PIKfyve 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 curve fitting 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 specificity of the anti-LC3 antibody as well validates the effective use of the autophagy enabling solutions.

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## Kit Components

- 20X Anti-LC3 FITC, clone 4E12: (Part No. CS208214). One vial containing 600  $\mu$ L

### CF200097: Autophagy Detection Reagent Pack (stored at 2°C - 8°C):

- 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

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## Materials Not Supplied

1. Test tubes for sample preparation and storage
2. Tissue culture reagents, i.e. HBSS, PBS w/o  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$ , cell dislodging buffers, etc.
3. Pipettors with corresponding tips capable of accurately measuring 10 – 1000  $\mu$ L
4. Tabletop centrifuge capable of achieving 300 x g
5. Mechanical vortex
6. Flow Cytometer
7. Deionized water (for reagent dilutions)
8. Earle's Balanced Salt Solution (EBSS)
9. Rapamycin reagent (EMD Chemicals; Part No. 553210)
10. Dynamin Inhibitor I, Dynasore reagent (EMD Chemicals; Part No. 324410)
11. PIKfyve Inhibitor (EMD Chemicals; Part No. 524611)
12. PI-103, ATP-competitive inhibitor of PI3-K and mTOR (EMD Chemicals; Part No. 528100)

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## 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.
- The conjugated antibody is light sensitive and must be stored in the dark at 2 - 8°C.
- Do not use reagents beyond the expiration date of the kit.

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## Storage

All reagents must be stored at 2 - 8°C.

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.**

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## 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 maintain them stored at -20°C. Avoid exposure of the reagent to repeated freeze and thaw cycles.*

### 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 - 8°C.

### 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 - 8°C

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## Assay Instructions

### I. General Assay Protocol (To monitor autophagosomes)

1. Seed cells of interest into a tissue culture flask overnight in a humidified 37°C incubator with 5% CO<sub>2</sub>.
2. If adherent cells, once the cells are 80% confluent aspirate and wash cells with 1X HBSS. Gently dislodge cells using a mild enzyme such as CDR Cell Dispersal Reagent (Cat No. 4700-0050) or Accutase, tap the sides of the flask to dislodge and harvest using growth media. Transfer cell culture to a centrifuge tube. Spin at 300 x g for 5 minutes at room temperature (RT) and aspirate media.
3. Resuspend cell pellet to 2x10<sup>5</sup> cells per milliliter in growth media and seed 200 µL (4x10<sup>4</sup> cells) into the desired wells of a 96-well plate. Incubate overnight in a 37°C incubator with 5% CO<sub>2</sub>.
4. Aspirate off media and wash wells with 200 µL of 1X HBSS.
5. For wells that are to be starved: add 200 µL of EBSS + 10 µL Autophagy Reagent A that have been diluted at a 1:50 dilution in EBSS (e.g. 2 µL Autophagy Reagent A into 98 µL EBSS). Then incubate at 37°C incubator for two to eight (2-8) hours to induce autophagy.
6. For wells that are to be left untreated: add 200 µL of growth media and incubate at 37°C incubator for the same time as the starvation samples (2-8) hours. This will serve as the control sample.
7. Following incubation, aspirate media and wash wells with 200 µL of 1X HBSS.

8. Add 100  $\mu\text{L}$  of a mild enzyme (or Accutase) into each well and incubate for 5 minutes in a 37°C incubator to detach cells.
9. Transfer cells to a “V” bottomed 96-well plate containing 100  $\mu\text{L}$  of 1X Assay Buffer (or centrifuge tube) and spin for 5 minutes at 300 x g.
10. Add 100  $\mu\text{L}$  of 1X Autophagy Reagent B to each well/tube and immediately spin at 300 x g for 5 minutes.
11. Aspirate supernatant and resuspend each sample in 95  $\mu\text{L}$  1X Assay Buffer + 5  $\mu\text{L}$  of 20X optimized anti-LC3/FITC antibody for 30 minutes at room temperature in the dark.
12. Wash once with 1X Assay Buffer to remove residual and unbound antibody and spin at 300 x g for 5 minutes.
13. Resuspend each well in 200  $\mu\text{L}$  of 1X Assay Buffer (or 500  $\mu\text{L}$  of 1x Assay Buffer in sample tubes (tubes) and acquire data.

*Note: Results are tested using Guava easyCyte systems. However, the kit can be used on any flow cytometer with the correct laser and filter configurations. Also, LC3 expression can vary depending on the cell line being evaluated. The kit is designed to measure endogenous levels of LC3, so be aware that some cell types may have very low endogenous levels of LC3 not measurable by immunodetection methods.*

## **II. General Protocol for Compound Screening (optional)**

### **a. Compound Hit Identification:**

1. Seed  $3 \times 10^4$  cells into a 96-well plate overnight in a humidified 37°C incubator with 5%  $\text{CO}_2$ . Cells should be at about 80-90% confluent the next day.
2. Dilute each compound to a final concentration 10  $\mu\text{M}$  in growth media preferred by the investigator (NOTE: Starting sample concentration must be determined at the researcher's own discretion).
3. Remove growth media from each well and wash once with 1X HBSS. Spin for 5 minutes at 200 x g.
4. Add 200  $\mu\text{L}$  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 at a 1:50 dilution in growth media (e.g. 2  $\mu\text{L}$  Autophagy Reagent A into 98  $\mu\text{L}$  growth media).
6. Add 10  $\mu\text{L}$  of the diluted Autophagy Reagent A (in step #5) to each well/sample.
7. Incubate the cells for 30 minutes at 37°C.

8. Aspirate media and wash cells once with 200  $\mu$ L of 1X HBSS.
9. Add 100  $\mu$ L of a mild enzyme (e.g. or Accutase) to each well.
10. Allow cells to incubate at 37°C for 5 minutes to detach cells.
11. During the incubation step, add 100  $\mu$ L of growth media to a guava compatible “V”-bottomed 96-well plate or centrifuge tube or centrifuge tube.
12. Gently resuspend cells in the 96-well plate to dislodge cells, followed by transferring cells to the “V”-bottomed 96-well plate (or centrifuge tube) for a total of 200  $\mu$ L in each well (500  $\mu$ L in each centrifuge tube).
13. Spin at 300 x g for 5 minutes at room temperature and discard supernatant.
14. Add 100  $\mu$ L of 1X Autophagy Reagent B to each well/tube and immediately spin at 300 x g for 5 minutes. Carefully discard supernatant from each well.
15. Resuspend cells in 95  $\mu$ L 1X Assay Buffer + 5  $\mu$ L of 20X optimized anti-LC3/FITC antibody for 30 minutes at room temperature in the dark.
16. Wash once with 1X Assay Buffer to remove residual and unbound antibody and spin at 300 x g for 5 minutes. Carefully discard supernatant from each well.
17. Resuspend cells in each well with 200  $\mu$ L 1X Assay Buffer.
18. Acquire samples.

## **b. Compound Dose Response Curves**

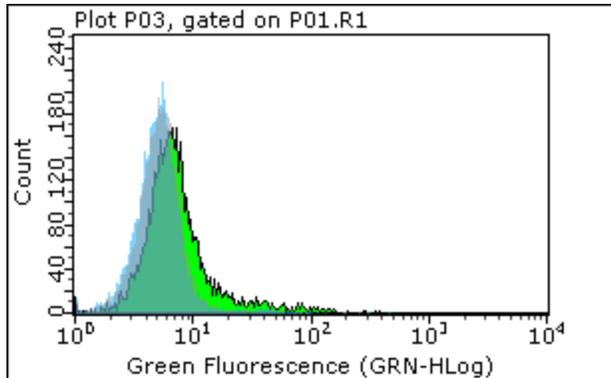
1. Seed  $3 \times 10^4$  cells into a 96-well plate overnight in a humidified 37°C incubator with 5% CO<sub>2</sub>. Cells should be at about 80-90% confluent the next day.
2. Dilute each compound to a final concentration of 10  $\mu$ M in growth media preferred by the investigator (NOTE: Starting sample concentration must be determined at the researcher's own discretion).
3. Serially 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  $\mu$ L 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 at a 1:50 dilution in growth media (e.g. 2  $\mu$ L Autophagy Reagent A into 98  $\mu$ L growth media).

7. When the desired time point is reached, add 10  $\mu\text{L}$  of the diluted Autophagy Reagent A (in step #6) to each well.
8. Incubate the cells for 30 minutes at 37°C.
9. Aspirate growth media and wash cells once with 200  $\mu\text{L}$  of 1X HBSS.
10. Add 100  $\mu\text{L}$  of a mild enzyme (e.g. or Accutase) to each well.
11. Allow cells to incubate at 37°C for 5 minutes to detach cells.
12. During the incubation step, add 100  $\mu\text{L}$  of growth media to a guava compatible “V”-bottomed 96-well plate or centrifuge tube.
13. Gently resuspend cells in the 96-well plate to dislodge cells, followed by transferring cells to the “V”-bottomed 96-well plate (or centrifuge tube) for a total of 200  $\mu\text{L}$  in each well (500  $\mu\text{L}$  in each centrifuge tube).
14. Spin at 300 x g for 5 minutes at room temperature and discard supernatant.
15. Add 100  $\mu\text{L}$  of 1X Autophagy Reagent B to each well/tube and immediately spin at 300 x g for 5 minutes. Carefully discard supernatant from each well.
16. Resuspend cells in 95  $\mu\text{L}$  1X Assay Buffer + 5  $\mu\text{L}$  of 20X optimized anti-LC3/FITC antibody for 30 minutes at room temperature in the dark.
17. Wash once with 1X Assay Buffer to remove residual and unbound antibody and spin at 300 x g for 5 minutes. Carefully discard supernatant from each well.
18. Resuspend cells in each well with 200  $\mu\text{L}$  1X Assay Buffer.
19. Acquire samples

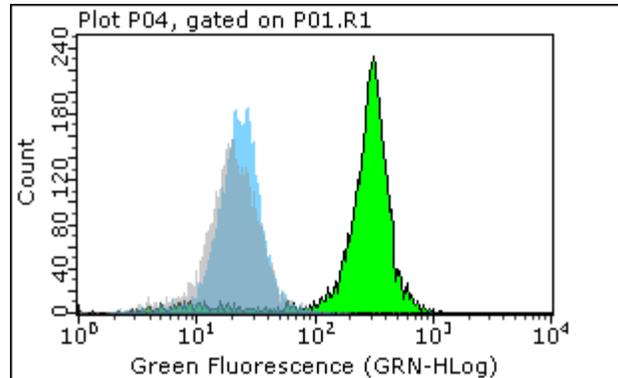
## Sample Data

### LC3 Antibody based Assay: Flow cytometry detection of LC3 translocation via autophagosomes by addition of a lysosome inhibitor (using anti-LC3/FITC conjugate)

#### A. Without Selective Permeabilization



#### B. With Selective Permeabilization



**Figure 3. Anti-LC3/FITC antibody for detecting the rate of autophagy and for drug screening on CHO cells.** In (A), without Selective Permeabilization no shift of LC3 level is detected using flow cytometry before and after starvation (induction of autophagy). The position of the histograms indicates the low endogenous LC3 expression and constitutive degradation.

In (B), with Selective Permeabilization, the shift of LC3 level is observed during autophagy only in the presence of lysosome inhibitor (green), which delays the LC3 degradation in the autophagosomes and prolongs the signal.

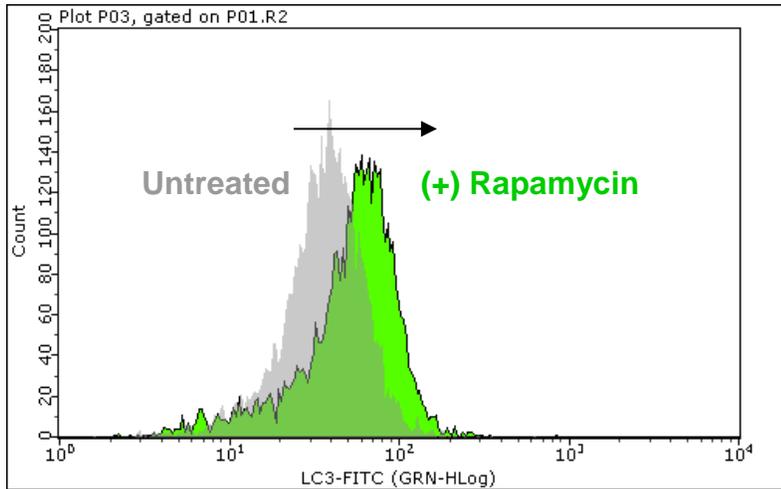
**Legend:**

Non-starved (control for no autophagy)

Starved in the absence of lysosome inhibitor

Starved in the presence of lysosome inhibitor

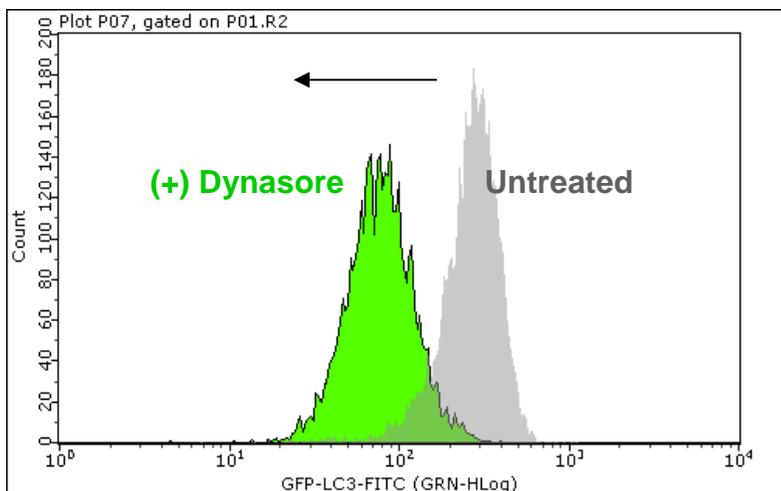
### Anti-LC3/FITC ( on CHO cells)



100 $\mu$ M CQ for 1 hr (control; No rapamycin)  
0.4 $\mu$ M Rapamycin 5hrs + 100 $\mu$ M CQ for 1 hr

**Figure 4. 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.

### Anti-LC3/FITC ( on HeLa cells)



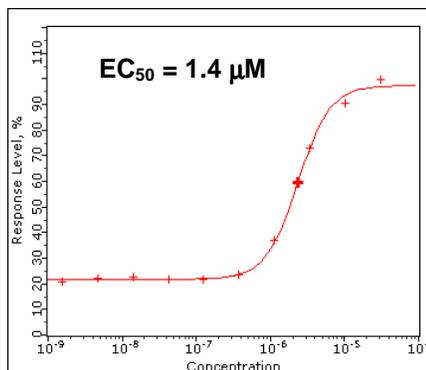
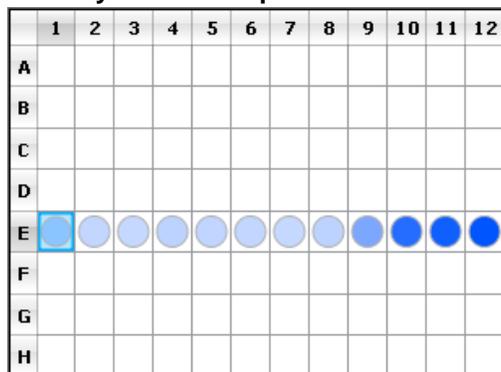
100 $\mu$ M CQ for 3 hrs (control; no Dynasore)  
80 $\mu$ M Dynasore + 100 $\mu$ M CQ for 3 hrs

**Figure 5. 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  $\mu$ M Dynasore for 3 hours prior to data acquisition.

**96-well Plate Heat map  
(12 pt, dose response assay)**

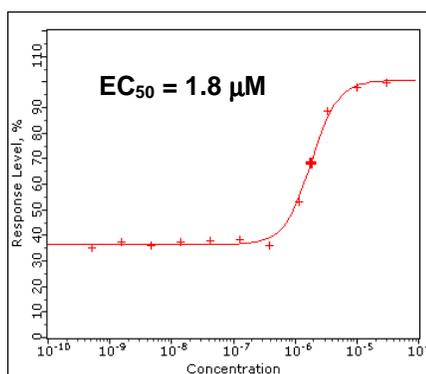
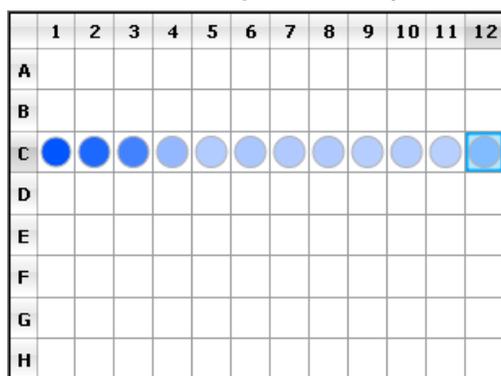
**Dose Response Curve**

**A. PIKfyve Dose response curve**



**PIKfyve**

**B. PI-103 Dose Response Assay**



**PI-103**

**Figure 6. Detailed small molecule evaluation by performing dose response curves by flow cytometry using the InCyte™ Software Module** PIKfyve and PI-103 have been indicated as autophagy compounds, and by using our selective permeabilization method along with the anti-LC3/FITC antibody conjugate we were able to utilize this assay as a viable screening tool. PIKfyve (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.
- For drug treatments, all incubation times and sample concentrations must be optimized at the researcher's own discretion. Some guidelines for drug treatment are provided in this kit but can be modified to suit the researcher's individual 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	<ul style="list-style-type: none"> <li>• If storing at -20°C, precipitate can form in the 10X wash buffer. Prior to use, place bottle in a 37°C water bath, swirling the contents occasionally. If this does not remove the precipitate completely, allow 10X Wash Buffer to sit at room temperature overnight.</li> </ul>
Acquisition	Acquisition rate decreases dramatically	<p>This usually indicates that the fluid pathway on the instrument may be blocked. This can be alleviated by the following:</p> <ul style="list-style-type: none"> <li>• Decreasing number of cells for analysis. Guava flow cytometers have the capacity of analyzing a steady stream of 300 – 500 cells per microliter. Using cell concentrations in excess of the recommended level 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 (5 x10<sup>5</sup> cells per ml).</li> <li>• Adherent cells can result in cellular clumping. Using a stronger enzyme for dissociation such as the guava ViaCount CDR Cell Dispersal Reagent (Cat. No. 4700-0050) or trypsin during cell harvesting should help keep cells in single suspension. Alternatively, using a cell strainer can help disrupt cell clumping if needed.</li> <li>• 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 stream takes place.</li> </ul>
Cellular Analysis	A loss or lack of signal	<ul style="list-style-type: none"> <li>• 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.</li> </ul>
Cellular Analysis	Variability in day to day experiments	<ul style="list-style-type: none"> <li>• 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.</li> <li>• 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)</li> </ul>

\*For Technical Service, please visit [www.millipore.com/techservice](http://www.millipore.com/techservice).

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## References

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2. Zhang, L., *et al.* (2007). Small molecule regulators of autophagy identified by an image-based high-throughput screen. *Proc Natl Acad Sci U S A*;104(48):19023-8.
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4. Mizushima, N., *et al.* (2004). Methods for monitoring autophagy. *Int J Biochem Cell Biol.*;36(12):2491-502.
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7. Turcotte, T., *et al.* (2008). A molecule targeting VHL-deficient Renal Cell Carcinoma that induces autophagy. *Cancer Cell*; 14(1):90-102.

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## Related Products

1. FlowCollect™ GFP-LC3 Reporter Autophagy Assay Kit (CHO) (Catalog No. FCCH100170)
2. FlowCollect™ GFP-LC3 Reporter Autophagy Assay Kit (U20S) (Catalog No. FCCH100181)
3. FlowCollect™ RFP-LC3 Reporter Autophagy Assay Kit (U20S) (Catalog No. FCCH100183)
4. FlowCollect™ Bivariate Cell Cycle Kit for DNA Replication Analysis (Catalog No. FCCH025102)
5. FlowCollect™ Bivariate Cell Cycle Kit for G2/M Analysis (Catalog No. FCCH025103)
6. FlowCollect™ Multi-Color DNA Damage Response Kit (Catalog No. FCCH025104)
7. FlowCollect™ Histone H2A.X Phosphorylation Assay Kit (Catalog No. FCCS100182)
8. FlowCollect™ Cell Cycle Checkpoint H2A.X DNA Damage Kit (Catalog No. FCCH025142)
9. FlowCollect™ Cell Cycle Checkpoint ATM DNA Damage Kit (Catalog No. FCCH025143)
10. FlowCollect™ PI3K-mTOR Signaling Cascade Mapping Kit (Catalog No. FCCS025210)
11. FlowCollect™ PI3K/MAPK Dual Pathway Activation and Cancer Marker Detection kit (Catalog No. FCCS025100)

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