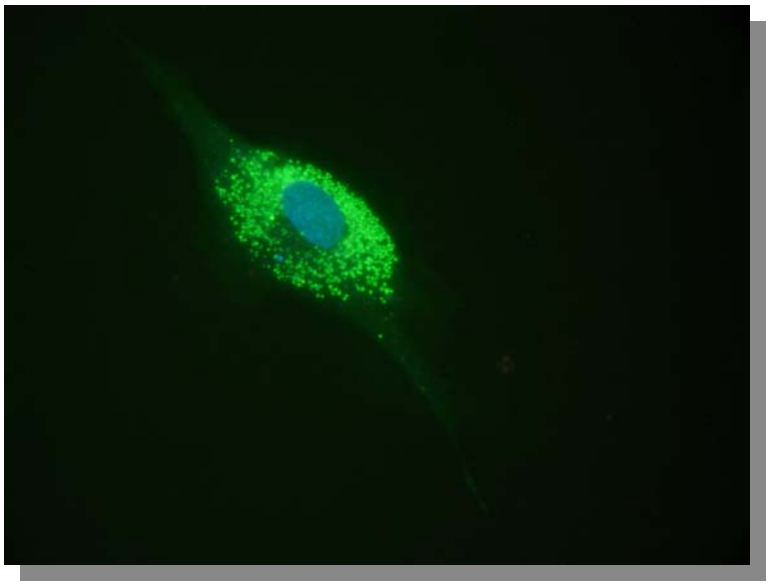
Product Information Sheet
(M1377-005)



MarkerGene™ LysoLive™ Lysosomal Sulfatase Assay Kit
(Product M1377)

Product Information Sheet
(M1377-004)
MarkerGeneTM LysoLiveTM
Lysosomal Sulfatase Assay Kit
(Product M1377)

NOTE: The following information is given as a viable methodology for use of MarkerGene™ LysoLive™ Lysosomal Sulfatase Assay Kit. The user may determine their own best conditions for use dependent on the specific conditions present in their experiment.

I. OVERVIEW

Lysosomes are acidic cytoplasmic organelles that are present in all nucleated mammalian cells. Lysosomes have been found to be involved in a variety of cellular processes including repair of the plasma membrane, defense against pathogens, cholesterol homeostasis, bone remodeling, metabolism, apoptosis, and cell signaling. Defects in lysosomal enzyme activity have been associated with a variety of diseases including Parkinson's, Tay-Sachs, Sandhoff, Krabbe, Wolman, and Gaucher syndromes. Marker Gene has developed lysosomal staining compounds that are useful for labeling lysosomes in a live-cell format and are capable of monitoring lysosomal metabolic activity. These new targeted substrates are based upon fluorescent probes that have a low pKa value for optimum fluorescence at the lower physiological pH values found in the lysosomes as well as targeting groups to direct their accumulation to the lysosomes using a live-cell staining format.

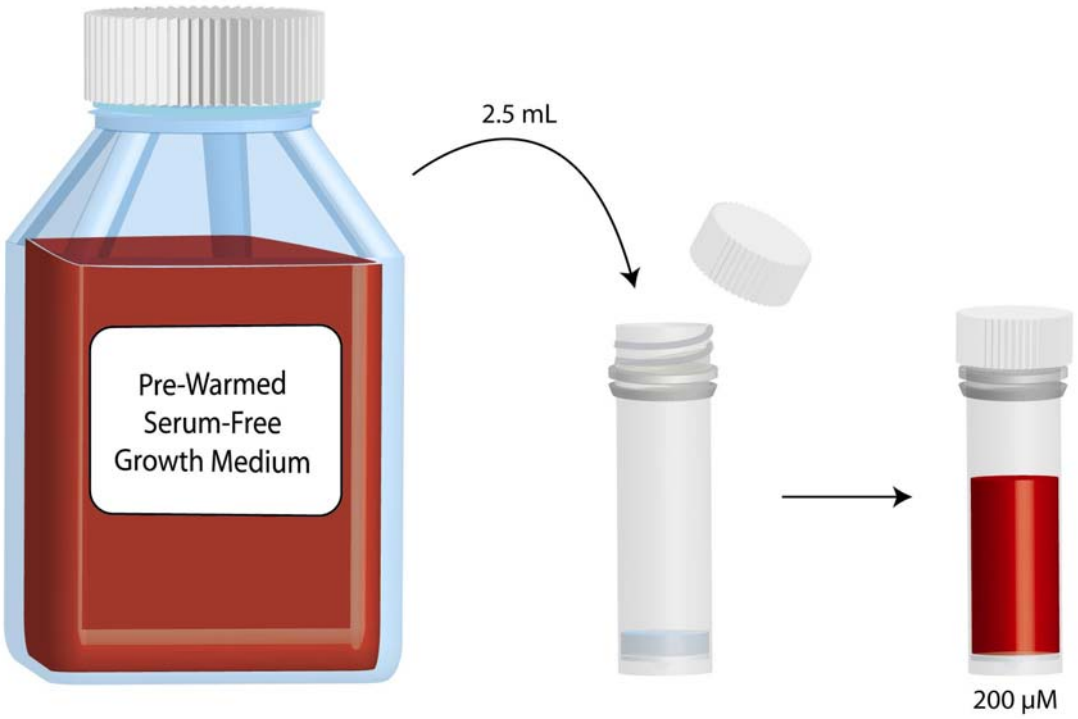
II. MATERIALS

A.) Lysosomal Substrate Reagent: 10mM LysoLive™ SulfGreen in DMSO (10 vials).

B.) Lysosomal Staining Standard: 5mg/mL Acridine Orange in DMSO (1 vial).

Storage and Handling: The Lysosomal Substrate Reagent and Lysosomal Staining Standard should be stored at -20˚C until needed. The Lysosomal Substrate Reagent is packaged in small aliquots for single use to avoid repeat freeze/thaw cycles. Both the Lysosomal Substrate Reagent and Lysosomal Staining Standard should be protected from light. Prolonged exposure of labeled cells to fluorescence lamps can result in photobleaching of the dyes.

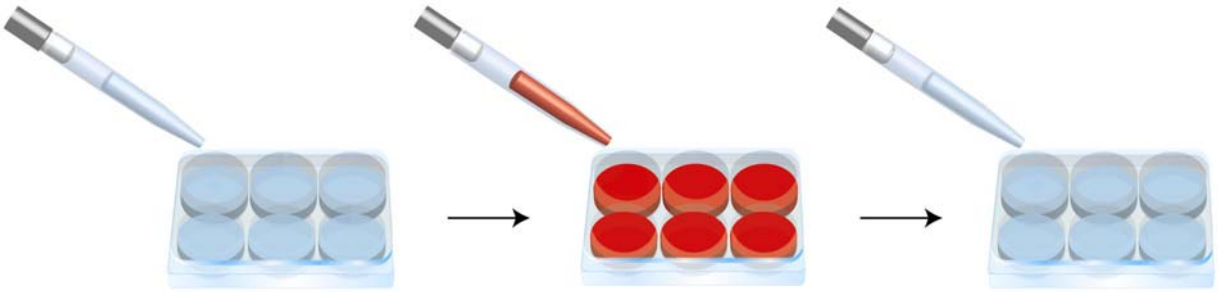
III. SUBSTRATE PREPARATION



All reagent preparation and cell staining procedures should be performed under sterile conditions, such as in a laminar flow hood.

Prepare staining medium by adding 2.5 mL pre-warmed (37˚C) growth medium (see Note (1) below) to one vial of Lysosomal Substrate Reagent (Product No. M1377-001) to yield a 200µM substrate solution. **This solution may be further diluted with growth medium to reach desired staining concentration**. (see Note (2)).

IV. CELL STAINING PROCEDURE



WASH CELLS WITH 1X PBS

ADD STAINING MEDIUM TO CELLS AND

WASH CELLS WITH 1X PBS AND VIEW/IMAGE

Cell Staining Protocol

1.) Remove growth medium from cells by suction.

2.) Wash cells with 1X Phosphate Buffered Saline (PBS). Remove by suction.

3.) Add staining medium to cells. See Table (1) for recommended volumes for different culture vessels.

4.) Place cells in incubator. Incubation times may range from 30 min. to 16 hours (see Note (2)).

5.) Remove staining medium by suction. Wash cells 3 times with 1X PBS.

6.) Cells may be mounted on slides for viewing (using epifluorescence microscopes) or viewed directly in the culture vessel (using inverted confocal microscopy). Users may determine their own best conditions for viewing cells.

7.) View cells using fluorescence filters with bandpass capable of visualizing fluorescence with EX/EM: 490/520 (such as Nikon B-2A, Omega Optical XF14-2, Omega Optical XF68).

Staining of cells with Lysosomal Staining Standard

1.) Prepare staining medium by adding Lysosomal Staining Standard (Product No. M1377-002) (5mg/mL) to unsupplemented pre-warmed growth medium. See Note (3).

2.) Remove growth medium from cells by suction.

3.) Wash cells with 1X Phosphate Buffered Saline (PBS). Remove by aspiration.

4.) Add staining medium to cells. See Table (1) for recommended volumes for different culture vessels.

5.) Place cells in incubator. Incubate cells for 10-30 mins.

6.) Remove staining medium by suction. Wash cells 3 times with 1X PBS.

7.) Cells may be mounted on slides for viewing (using epifluorescence microscopes) or viewed directly in the culture vessel (using inverted confocal microscopy). Users may determine their own best conditions for viewing cells.

8.) View cells using fluorescence filters with bandpass capable of visualizing long wavelength fluorescence (i.e. Texas Red filters, such as Omega Optical XF40) See Note (4).

NOTE (1): It is recommended that the Lysosomal Substrate Reagent be diluted in serum-free growth medium appropriate for the cell line to be assayed. Use of serum may produce inaccurate results due to exogenous enzyme activities present in serum. It is also suggested that medium used for staining be free of antibiotics/antimycotics, to avoid any potential effects of these compounds on enzyme activities within the cells.

NOTE (2): It is recommended that users determine their own optimum staining concentrations and incubation times. Optimum conditions can vary greatly depending on cell line, culture conditions, and sensitivity of fluorescence microscopy equipment. It is suggested that users stain cells with the highest recommended concentration of substrate (200µM) and/or standard, and scale down accordingly if staining is too intense.

NOTE (3): It is recommended that users determine their own optimum staining concentrations when using the lysosomal staining standard. Optimum conditions can vary greatly depending on cell line, culture conditions, and sensitivity of fluorescence microscopy equipment. It is suggested that users stain cells at a concentration of 5µg/mL lysosomal staining standard, and scale up/down accordingly if staining is too weak/intense.

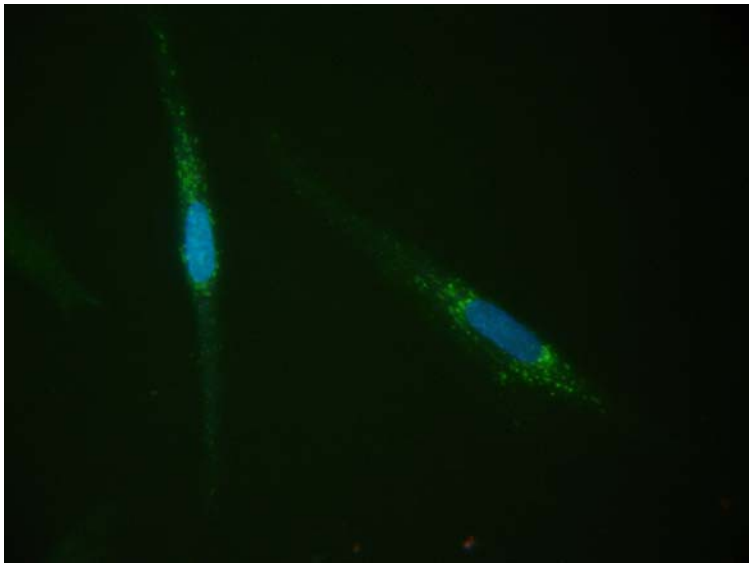
NOTE (4): Lysosomal staining standard labels lysosomes with a red fluorescence, while the nuclei and cytoplasm of cells will be labeled with a green fluorescence. Thus, a filter capable of visualizing long-wavelength fluorescence is required for viewing lysosomal staining.

TABLE (1): RECOMMENDED REAGENT VOLUMES FOR DIFFERENT CULTURE VESSELS.

Culture Vessel	**Rec. Vol. Staining Medium**	**Rec. Vol. 1X PBS**
100MM CULTURE DISH	10 ML	5 ML
60MM CULTURE DISH	4 ML	2 ML
6-WELL CULTURE PLATE	2 ML/WELL	1 ML
12-WELL CULTURE PLATE	1 ML/WELL	0.5 ML
24-WELL CULTURE PLATE	0.5 ML/WELL	0.25 ML
96-WELL CULTURE PLATE	200 ML/WELL	100 ML

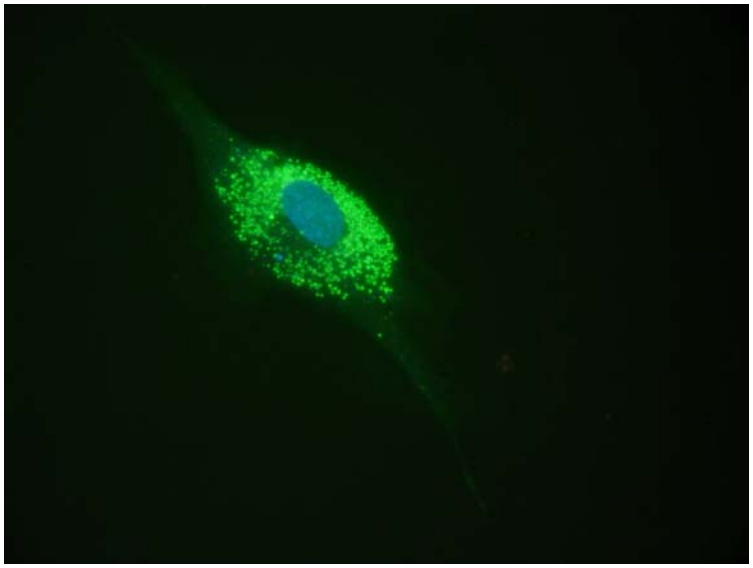
Figure 1: Labeling cells with MarkerGene™ LysoLive™ Lysosomal Sulfatase Assay Kit

A.)



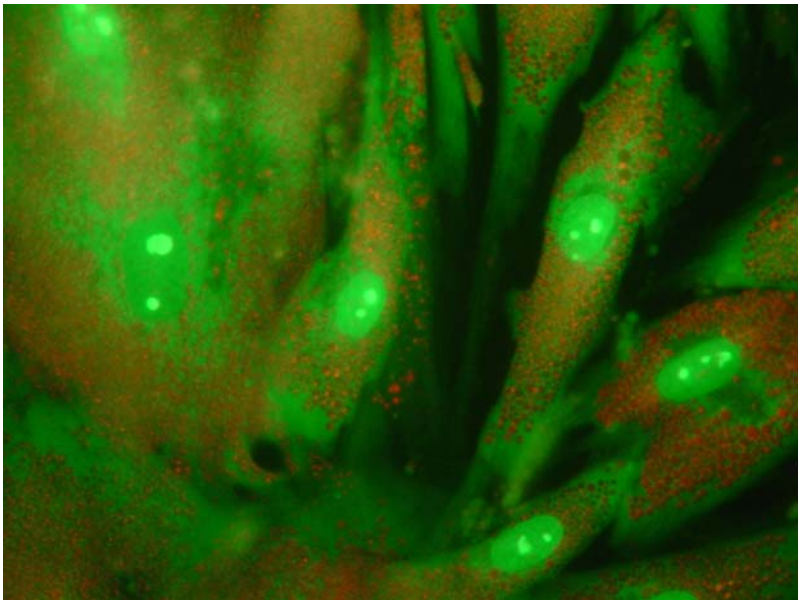
Human Fibroblast (healthy donor, **GM03440**)
200µM LysoLive™ SulfGreen, Overnight Incubation
XF68 multi-band filter (Omega Optical), 2s shutter speed

B.)



GM03440 60 h. incubation in 100mM sucrose
200µM LysoLive™ SulfGreen, Overnight incubation
XF68 multi-band filter (Omega Optical), 2s shutter speed

Figure 2: Labeling cells with Lysosomal Staining Standard (M1377-002)



Human Fibroblast (healthy donor, **GM03440**)
5µg/mL Lysosomal Staining Standard (M1377-002), 30 min. incubation
XF68 multi-band filter (Omega Optical), 1s shutter speed

M1377 KIT CONTENTS			
DESCRIPTION	**QUANTITY**	**PART NO.**	**STORAGE**
REAGENTS			
LYSOSOMAL SUBSTRATE REAGENT- LysoLive™ SulfGreen	10 X 50ML VIAL	1377-001	F,L,T,R
LYSOSOMAL STAINING STANDARD	1 X 100ML VIAL	1377-002	F,L,R
DOCUMENTATION			
MSDS SHEETS	2	1377-003/4	N/A
PRODUCT INFORMATION SHEET	1	1377-005	N/A

Notes: F=store at or below -20°C; C=store cold (4°C); L=light sensitive; T=avoid repeat freeze/thaw; R=read protocol;instructions carefully prior to use.

REFERENCES

1.) Carpenter AE,Jones TR, Lamprecht MR, Clarke C, Kang IH, Friman O, Guertin DA, Chang JH, Lindquist RA, Moffat J, Golland P, Sabatini DM (2006) "CellProfiler: image analysis software for identifying and quantifying cell phenotypes." Genome Biology 7:R100.

2.) Karageorgos LE, Isaac EL, Brooks DA, Ravenscroft EM, Davey R, Hopwood JJ, Meikle PJ (1997) "Lysosomal Biogenesis in Lysosomal Storage Disorders" Experimental Cell Research 234, 85-97.

3.) Koenig H (1963) "Vital staining of lysosomes by acridine orange." J. Cell Biol 19, 87A.

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