

Uptake of AcidiFluor™ ORANGE-anti-EGFR antibody by A431 cell (EGFR overexpressing cell line)

YouTube GoryoChemical : <http://www.youtube.com/watch?v=NB7VuuRYXRM>

Phagocytosis of AcidiFluor ORANGE™- Zymosan A by RAW264.7

YouTube GoryoChemical : <http://www.youtube.com/watch?v=Vdprn0MmPRE>

AcidiFluor™ Series

Fluorescence imaging probe to detect acidic environment

Code no.	Product	Size	Remarks
GC301	AcidiFluor™ ORANGE	10 µg × 20	A fluorogenic probe for acidic organelles in living cells.
GC3011	AcidiFluor™ ORANGE	10 µg × 10	A fluorogenic probe for acidic organelles in living cells (trial size).
GC302	AcidiFluor™ ORANGE-NHS	1 mg	A pH indicator that can be conjugated to protein or other amine-containing compounds efficiently.
GC303	AcidiFluor™ ORANGE-NHS	5 samples	A pH indicator that can be conjugated to protein or other amine-containing compounds efficiently (small quantity).
GC304	AcidiFluor™ ORANGE Labeling Kit	5 samples	A labeling kit of AcidiFluor™ ORANGE including all reagents to label protein or other amine-containig compounds efficiently.
GC305	AcidiFluor™ ORANGE-Zymosan A	1 mg	Fluorescent reagent for analyzing phagocytosis.
GC306	AcidiFluor™ ORANGE-Dextran 10K	1 mg	Fluorescent reagent for detecting endocytosis.
GC307	AcidiFluor™ ORANGE-Beads 500	500 nm /250 µg	AcidiFluor™ ORANGE fixed organic nanosilica for detecting endocytosis.
GC3071	AcidiFluor™ ORANGE-Beads 1000	1 µm /250 µg	AcidiFluor™ ORANGE fixed organic nanosilica for detecting endocytosis.
GC308	AcidiFluor™ ORANGE-wBeads 500	500 nm /250 µg	AcidiFluor™ ORANGE fixed and FITC-included organic nanosilica for detecting endocytosis.
GC3081	AcidiFluor™ ORANGE-wBeads 1000	1 µm /250 µg	AcidiFluor™ ORANGE fixed and FITC-included organic nanosilica for detecting endocytosis.
GC309	AcidiFluor™ ORANGE-Transferrin	1 mg	For analyzing receptor-mediated endocytosis.
GC310-01	HaloTag® AcidiFluor™ ORANGE Ligand	30 nmol	pH sensor combined with Halotag Ligand. For research of exocytosis and endocytosis.

References

1. Hayashi A, Asanuma D, Kamiya M, Urano Y, Okabe S. High affinity receptor labeling based on basic leucine zipper domain peptides conjugated with pH-sensitive fluorescent dye: visualization of AMPA type glutamate receptor endocytosis in living neurons. Neuropharmacology. 2015 Jul 25. pii: S0028-3908(15)30033-2. doi: 10.1016/j.neuropharm.2015.07.026.
2. Asanuma D, Takaoka Y, Namiki S, Takikawa K, Kamiya M, Nagano T, Urano Y & Hirose K. Acidic-pH-Activatable Fluorescence Probes for Visualizing Exocytosis Dynamics. Angew Chem Int Ed 2014, doi:10.1002/anie.201402030.
3. Masayuki Isa, Daisuke Asanuma, Shigeyuki Namiki, Kazuo Kumagai, Hirotatsu Kojima, Takayoshi Okabe, Tetsuo Nagano, and Kenzo Hirose, ACS Chem Biol 2014 Oct 22;9(10):2237-41, "High-throughput screening system to identify small molecules that induce internalization and degradation of HER2."
4. Watanabe R, Soga N, Fujita D, Tabata KV, Yamauchi L, Kim SH, Asanuma D, Kamiya M, Urano Y, Suga H and Noji H. Nature Communications 2014 Jul24; 5, Article number: 4519 doi:10.1038/ncomms5519 "Arrayed lipid bilayer chambers allow single-molecule analysis of membrane transporter activity"

Distributor

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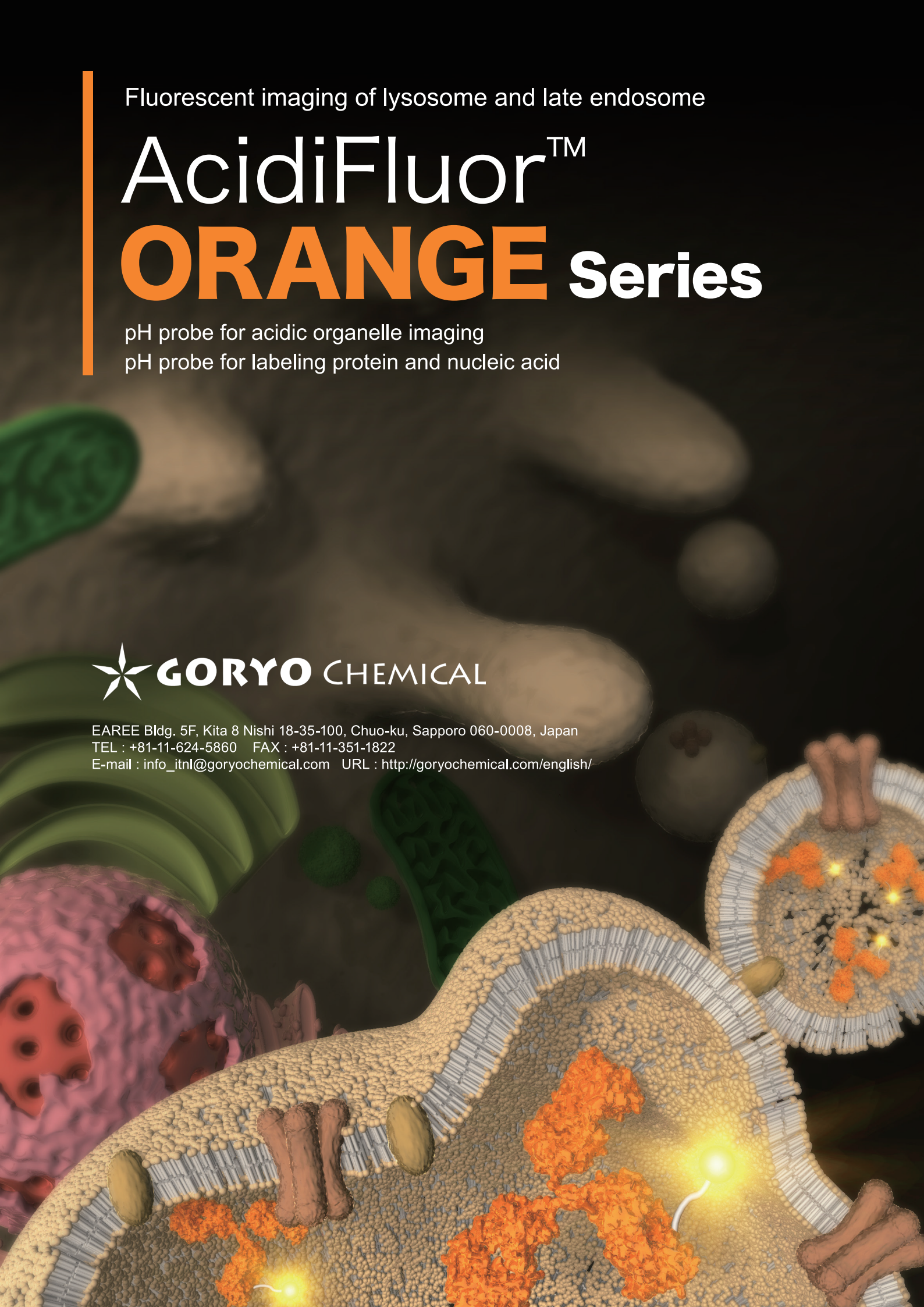
Fluorescent imaging of lysosome and late endosome

AcidiFluor™
ORANGE Series

pH probe for acidic organelle imaging
pH probe for labeling protein and nucleic acid



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Fluorescent imaging of lysosome and acidic organelles

AcidiFluor™ ORANGE

- High S/N ratio
- Orange color fluorescence and usable for multicolor imaging
- Great photostability

AcidiFluor™ ORANGE is a fluorescence imaging probe which enhances fluorescence dramatically in acidic environments. This probe can stain acidic organelles such as lysosome, late endosome and granule selectively.

Its excellent selectivity enables detection of acidic environments. Namely, in the condition of pH 5.0, identical to the environment of acidic organelles, fluorescence intensity is 50-fold or more compared to that of physiological pH 7.4. AcidiFluor™ ORANGE shows great photostability against irradiating excitation light.

As it emits orange color fluorescence by excitation at 532 nm or 514 nm, multicolor imaging is possible by combining with blue fluorescence (CFP, Hoechst 33342, etc.), green fluorescence (GFP, fluorescein, etc.) and near-infrared fluorescence.

AcidiFluor™ ORANGE can be used for detecting acidic organelles, observing granule release and imaging of endocytosis / exocytosis.

Feature 1 High S/N ratio

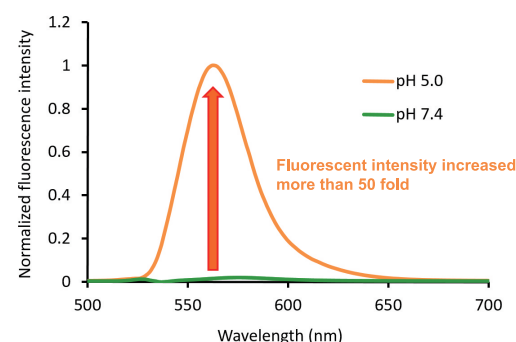


Fig.1 Fluorescence spectra of AcidiFluor ORANGE™ as a function of pH.

Fluorescence spectra of AcidiFluor™ ORANGE was measured in phosphate buffer pH 5.0 or pH 7.4, respectively. pH 5.0 buffer corresponds to the condition in the acidic organelles, and pH 7.4 corresponds to the physiological cytoplasmic conditions. Fluorescent intensity of AcidiFluor™ ORANGE at pH 5.0 increased 50-fold to that in the pH 7.4 buffer. (λ_{ex} 532 nm / λ_{em} 568 nm)

Feature 2 Orange color fluorescence suitable for multicolor imaging

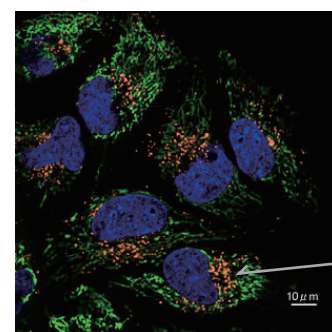


Fig.2 Example of multicolor imaging using HeLa cell

HeLa cell expressed Mitochondria-GFP was stained along with AcidiFluor™ ORANGE and Hoechst 33342 to enable multicolor imaging. Lysosomes were stained orange with AcidiFluor™ ORANGE, nucleuses were stained blue with Hoechst 33342 and mitochondria were stained green with GFP. AcidiFluor™ ORANGE can be useful for multicolor imaging.

Feature 3 Great photostability

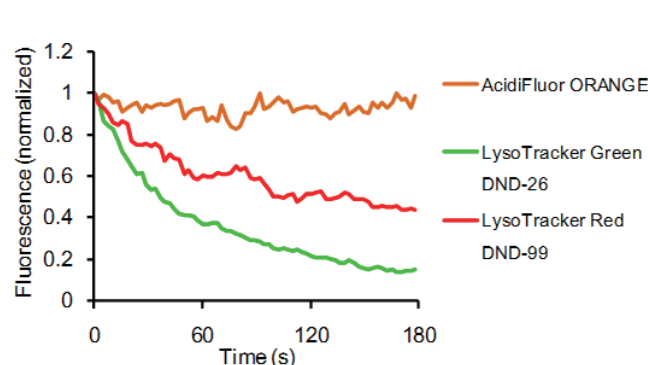


Fig.3 Comparison to competitor's products by photocleaching test

Samples were continuously illuminated for 180 seconds and viewed by confocal microscopy. Although LysoTracker® Green DND-26 and LysoTracker® Red DND-99 bleaches quickly, fluorescence of AcidiFluor™ ORANGE remained. LysoSensor™ Green DND-189 was not suitable for continuous observation because fluorescence leaked to cytoplasm by excitation light irradiation.

Monitoring pH under local environment and detecting endocytosis / phagocytosis

AcidiFluor™ ORANGE-NHS

- Just mixing
- High S/N ratio
- High responsivity after labeled to protein

AcidiFluor™ ORANGE-NHS is pH probe for labeling protein and nucleic acid.

All you have to do is mixing AcidiFluor™ ORANGE-NHS with antibody (pretein). Protein forms stable covalent bond through the intermediary of N-hydroxysuccinimide ester (NHS). This product can be used for monitoring pH of local environment, or detecting endocytosis / phagocytosis.

AcidiFluor™ ORANGE-NHS even after labeling with protein has excellent selectivity which enables detection of acidic environment. Namely, in the condition of pH 5.0, identical to the environment of acidic organelles, fluorescence intensity is 10-fold or more compared to that of physiological pH 7.4. AcidiFluor™ ORANGE shows great photostability against irradiating excitation light.

As it emits orange color fluorescence by excitation at 544 nm, multicolor imaging is possible by combining with blue fluorescence (CFP, Hoechst 33342, etc.), green fluorescence (GFP, fluorescein, etc.) and near-infrared fluorescence. Furthermore, not only microscope imaging but evaluating of uptake the compounds using fluorescence plate reader is possible.

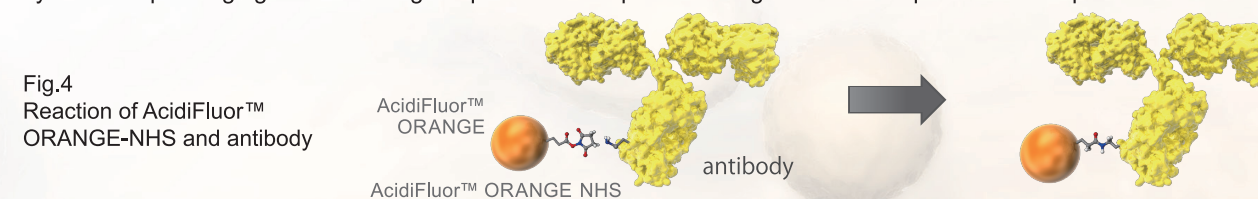


Fig.4 Reaction of AcidiFluor™ ORANGE-NHS and antibody

Feature High S/N ratio

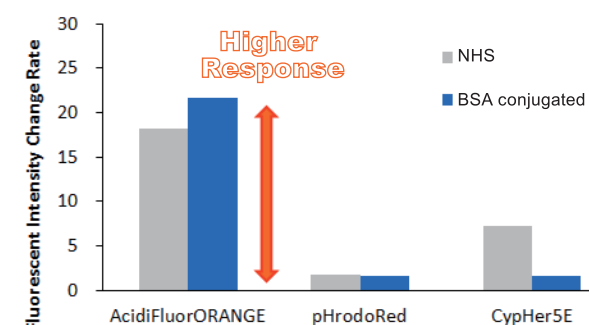


Fig.5 Comparative change in fluorescent intensity

Fluorescent intensity of AcidiFluor™ ORANGE-NHS as the BSA labeled form were compared with competitor probes in phosphate buffer pH 5.0 or pH 7.4. Intensity of AcidiFluor™ ORANGE-NHS in pH 5.0 increased 20 fold compared to pH 7.4, while other probes showed only 1.8 fold or 7.5 fold increase in fluorescent intensity. Only AcidiFluor™ ORANGE-NHS has high pH responsivity and dynamic range as both the NHS and BSA labeled forms.

AcidiFluor™ ORANGE-NHS : λ_{ex} 532 nm / λ_{em} 568 nm
pHrodo™ Red-NHS (Life Technology) : λ_{ex} 560nm / λ_{em} 582 nm
CypHer 5E-NHS (GE Healthcare) : λ_{ex} 644 nm / λ_{em} 667 nm

AcidiFluor™ ORANGE-NHS Application 1

Uptake of AcidiFluor™ ORANGE-Transferrin by HeLa cell

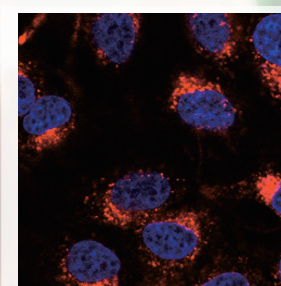


Fig.7 Uptake of AcidiFluor™ ORANGE-Transferrin by HeLa cell

HeLa cell was treated with AcidiFluor™ ORANGE-Transferrin, incubated at 37 °C for 3 hours, then observed by using confocal microscope. Fluorescent-Transferrin signal derived from AcidiFluor™ ORANGE was detected around a nuclei. This result means that AcidiFluor™ ORANGE-Transferrin was taken up by the cells, and migrated to lysosome.

AcidiFluor™ ORANGE-NHS Application 2

Uptake of AcidiFluor™ ORANGE-anti-EGFR antibody by A431 cell (EGFR overexpressing cell line)

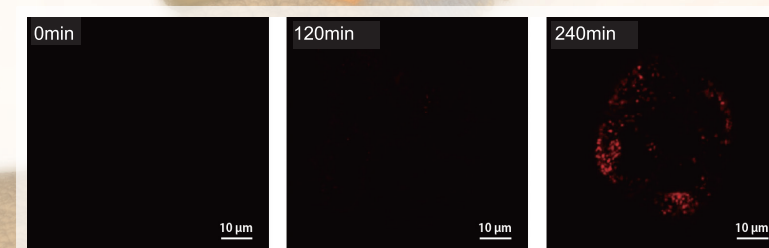


Fig.8 Uptake of AcidiFluor™ ORANGE-anti-EGFR antibody by A431 cell (EGFR overexpressing cell line)

Anti-EGFR antibody was labeled with AcidiFluor™ ORANGE-NHS, and unreacted dye was removed by ultrafiltration. EGFR overexpressing cell line was treated with purified AcidiFluor™ ORANGE-anti-EGFR antibody, then was observed by using confocal microscope. After 120min of addition, fluorescent signal derived from AcidiFluor™ ORANGE began to be detected, and further 60 min later, strong fluorescence was observed. This result means that AcidiFluor™ ORANGE-anti-EGFR antibody was taken up by the cell, and migrated to lysosome.