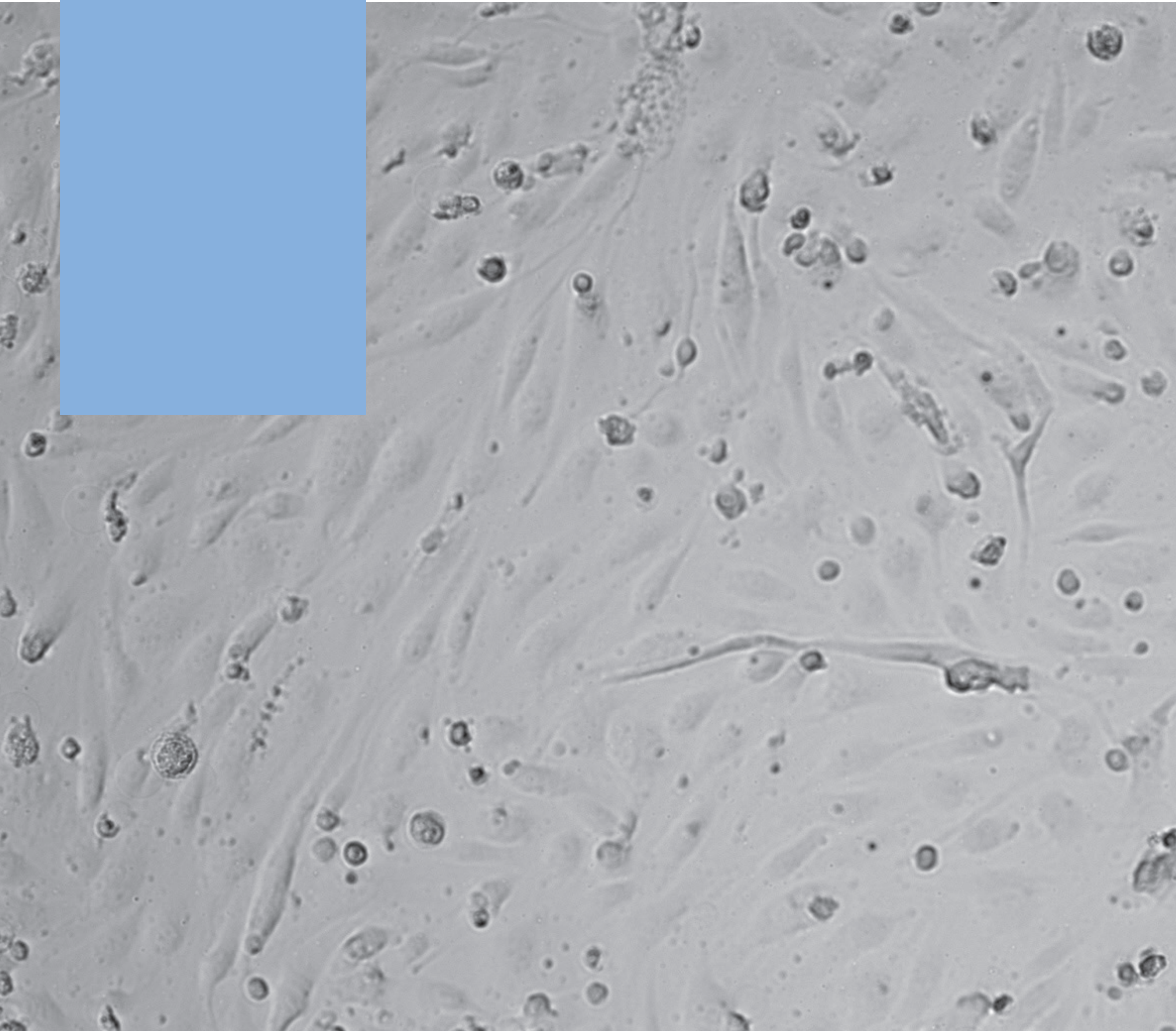
BioResearch

Lonza

Clonetics™ primary sensors –
HMVEC-L calcium biosensor



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1 Introduction

In order to provide primary cells for highly sensitive calcium assays, Clonetics™ HMVEC-L have been transiently transfected with the i-Photina® calcium-dependent photoprotein, a mutant of the jellyfish (*clytia gregarium*) photoprotein clytin (see references 1–4). Before freezing, the cells were loaded with the photoprotein's substrate coelenterazine.

The cells only have to be thawed and are ready-to-use for screening compounds triggering calcium release from internal stores.

1.1 Endothelial cells

Endothelial cells build up the thin layer lining the interior surface of blood and lymph vessels. These cells form an interface between the circulating fluid in the lumen and the outer layers of the vessel wall. Therefore endothelial cells can be found in the entire circulatory system, from the heart to the microvasculature. They are involved in many aspects of vascular biology, including the control of blood pressure through vasoconstriction and vasodilation, artherosclerosis, angiogenesis, blood clotting (thrombosis and fibrinolysis), inflammation and barrier function. This selective barrier function between the vessel lumen and the surrounding tissue controls the passage of materials and the transit of white blood cells into and out of the vessel. Excessive or prolonged increases in permeability of the endothelial cell monolayer, as in cases of chronic inflammation, may lead to tissue swelling.

These diverse functions disclose the indispensable roles of endothelial cells in the body's normal homeostasis and in many pathological conditions (see reference 5).

HMVEC-L are human microvascular endothelial cells of the lung. These large flat adherent cells grow in confluent monolayer.

1.2 Calcium signaling

Calcium (Ca^{2+}) has been known for a long time as an intracellular second messenger, playing an important role in many signaling pathways downstream of G-protein coupled receptors (GPCR, the major target class for drugs currently on the market), receptor tyrosine kinases and in ion-channel activity.

Calcium signaling in endothelial cells is a very interesting research area as most endothelial cell functions in the human body depend to various extents on changes in intracellular calcium concentration. Especially store-operated calcium entry is one of the strongest regulators of endothelial cell functions.

HMVEC-L are a relevant cell type for studies in the area of inflammatory processes, for example atherosclerosis, bronchial asthma and pulmonary fibrosis. Important mediators at the level of immune responses in such diseases are cysteinyl leukotrienes like LTC4 and LTD4. These compounds induce calcium release from internal stores upon stimulation of their specific receptors (CysLTR1+CysLTR2) expressed on these cells. Therefore, calcium signalling in HMVEC-L is investigated in vascular inflammatory diseases.

Mechanism of a calcium-dependent photoprotein

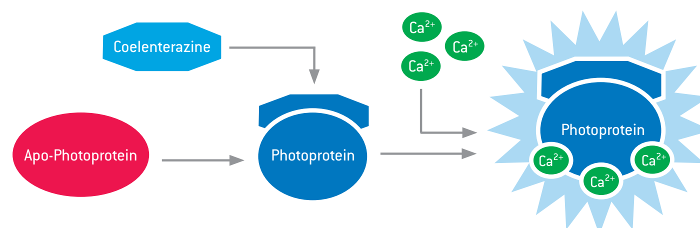


Figure 1. The i-Photina® calcium dependent photoprotein is expressed as an inactive apo-photoprotein. Upon binding of the substrate coelenterazine the active complex is formed. Stimulation of cells via Gαq-Protein coupled receptors induces calcium release from internal stores. Binding of calcium to the complex causes a conformational change to an excited state. The following rapid reaction results in a luminescence light flash which can be detected by a photo-multiplier in a plate reader.

2 Exemplary data

Signal kinetic of HMVEC-L calcium biosensor

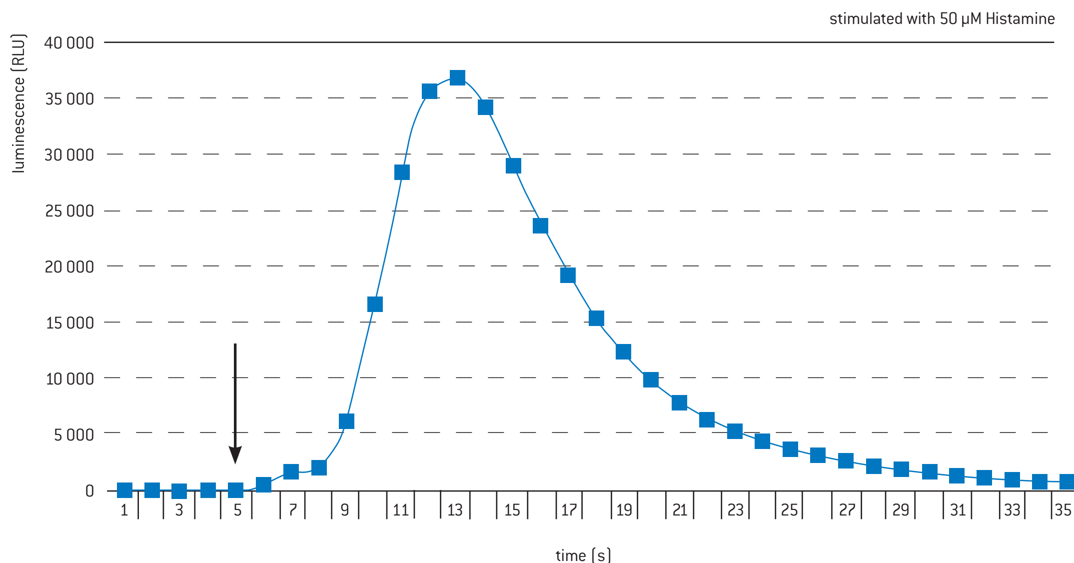


Figure 2. Cells were stimulated with 50 µM histamine (Sigma Aldrich, Cat. No. H7125) after reactivation from frozen state. Luminescence was recorded using a luminescence plate reader equipped with injection device. Arrow indicates the time point of histamine injection. The signal rises rapidly to maximum within 5–15 seconds.

3 Product description

3.1 Kit content

Cat.No.	CS-HML01A
Size	sufficient for one microplate
HMVEC-L calcium biosensor	1 cryo-amp
EBM™-2 basal medium	500 ml (Lonza, Cat No. cc-3156)
EGM™-2 MV SingleQuots™ supplements and growth factor	1 package (Lonza, Cat No. CC-4147)

Cat.No.	CS-HML01B
Size	sufficient for 1/4 microplate
HMVEC-L calcium biosensor	1 cryo-amp
EBM™-2 basal medium	500 ml (Lonza, Cat No. cc-3156)
EGM™-2 MV SingleQuots™ supplements and growth factor	1 package (Lonza, Cat No. CC-4147)

3.2 Storage and stability

- Remove cryovials from the dry shipper and immediately store in liquid nitrogen. Alternatively, thaw and use the cells immediately.
- **BulletKit™ instructions:** Upon arrival, store basal medium at 4–8°C and SingleQuots™ at -20°C in a freezer that is not self-defrosting. If thawed upon arrival, growth factors can be stored at 4°C and added to basal medium within 72 hours of receipt. After SingleQuots™ are added to basal medium, use within one month. Do not re-freeze.

3.3 Safety statements

These products are for research use only.
Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

Warning: Clonetics™ and Poietics™ products contain human source material. Treat as potentially infectious.

Each donor is tested and found non-reactive by an FDA approved method for the presence of HIV-I, Hepatitis B Virus and Hepatitis C Virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, Hepatitis B Virus, and Hepatitis C Virus. Testing can not offer complete assurance that HIV-1, Hepatitis B Virus, and Hepatitis C Virus are absent. All human sourced products should be handled in accordance to local country specific requirements but at least biological safety level 1 is strongly recommended. If you require further information, please contact your site safety officer or scientific support.

4 Assay protocol

4.1 Required material

Materials to be supplied by the user:

1M HEPES buffer, pH 7.2 (Lonza, Cat. No. 17-737E)
96-well solid white flat bottom polystyrene TC-treated microplates (e.g. Corning, Cat. No. 3917)
Compounds of interest for stimulation
Luminescence plate reader equipped with injection device
25°C incubator (can be without CO_2)

4.2 Preparation of media

NOTE: Volumes calculated for 96-well format. Adjust plating volume of cell suspension when using other microplate formats! One test vial is sufficient for 1/4 microplate.

- Decontaminate the external surfaces of all supplement vials and the medium bottle with ethanol or isopropanol.
- Aseptically open each supplement vial and add the entire amount to the basal medium with a pipette.
- Rinse each cryovial with the medium. It may not be possible to recover the entire volume listed for each cryovial. Small losses, even up to 10%, should not affect the cell growth characteristics of the supplemented medium.
- Transfer the label provided with each kit to the basal medium bottle being supplemented. Use it to record the date and amount of each supplement added. We recommend that you place the completed label over the basal medium label (Avoid covering the basal medium lot and expiration date) to avoid confusion or possible double supplementation.
- Record the new expiration date on the label based on the shelf life.

NOTE: If there is concern that sterility was compromised during the supplementation process, the entire newly prepared growth medium may be refiltered with a 0.2 µm filter to confirm sterility. Routine refiltration is not recommended.

4.3 Thawing the cells and performing the calcium assay

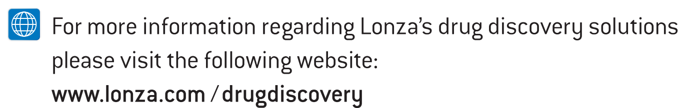
- Prewarm 26 ml (6.5 ml for test vial) of EGM™-2 MV complete medium to 25°C.
- Transfer cryo-amp with cells from liquid nitrogen onto ice for transport.
- Thaw the cells by placing the cryo-amp in a 37°C waterbath for not more than 2 minutes. When the last sliver of ice melts, immediately remove it from the waterbath.
- Carefully rinse the vial with ethanol. Wipe off remaining ethanol with paper towel or tissue.
- Add 700 µl of the prewarmed EGM™-2 MV to the cryo-amp and **gently mix cell suspension** with medium by pipetting up and down **once**.
- Transfer cell suspension to the tube with prewarmed media, use 1 ml of this suspension to rinse cryo-amp once.
- Transfer cell suspension to a pipetting reservoir.
- Plate cells in a 96-well plate (see required material): 250 µl per well using an 8-channel pipette. Take care that the cells don't settle in the pipetting reservoir during the plating procedure by **pipetting cell suspension up and down once** before each pipetting step. **Avoid air bubbles during pipetting. We recommend pipetting triplicates within one pipetting step!**
- Leave the plated cells on a bench-top (not in a flow-hood) in the dark for 1 hour to allow cells to settle.
- Transfer to a humidified tissue-culture incubator with 5% CO_2 / 37°C and incubate the cells for 3 hours.

NOTE: When looking through the microscope at this point some debris may appear in the culture. This is due to the various treatments of the cells right before freezing and does not affect the performance of the assay. The debris is usually removed by the media exchange.

- Prepare 20 ml of EGM™-2 MV complete medium supplemented with 20 mM HEPES, pH 7.2: Add 400 µl 1M HEPES to 19.6 ml EGM™-2 MV complete medium.
- Prewarm the HEPES buffered complete medium to 37°C.
- Carefully remove all the media from the cells with an 8-channel pipette and add 120 µl per well fresh prewarmed EGM™-2 MV complete medium supplemented with 20 mM HEPES.
- Transfer to a tissue-culture incubator with 5% CO_2 / 37°C and incubate overnight.
- Place the plate in a 25°C incubator for 1 hour (can be without CO_2).
- Prepare your compound dilutions.
- Volume of the compound to be injected should be 50 µl per well.
- Inject the compound and record the flash luminescence signal for 30–60 seconds.

NOTE: Primary HMVEC-L express the photoprotein only transiently. Agonist stimulation is mediated through endogenous receptors and signalling pathways expressed at physiological levels. Therefore, signal intensity is expected to be lower (around by a factor of 10) than with recombinant cell lines stably expressing either a photoprotein or a receptor at high level. So, you may need to adjust the sensitivity of your reader.

5 Additional information



For more information regarding Lonza's drug discovery solutions please visit the following website:
www.lonza.com / drugdiscovery

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