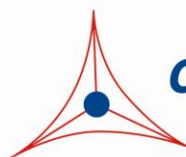

Product Manual

CytoSelect™ 96-Well Cell Migration Assay (5 µm, Fluorometric Format)

Catalog Number

CBA-105	96 assays
CBA-105-5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

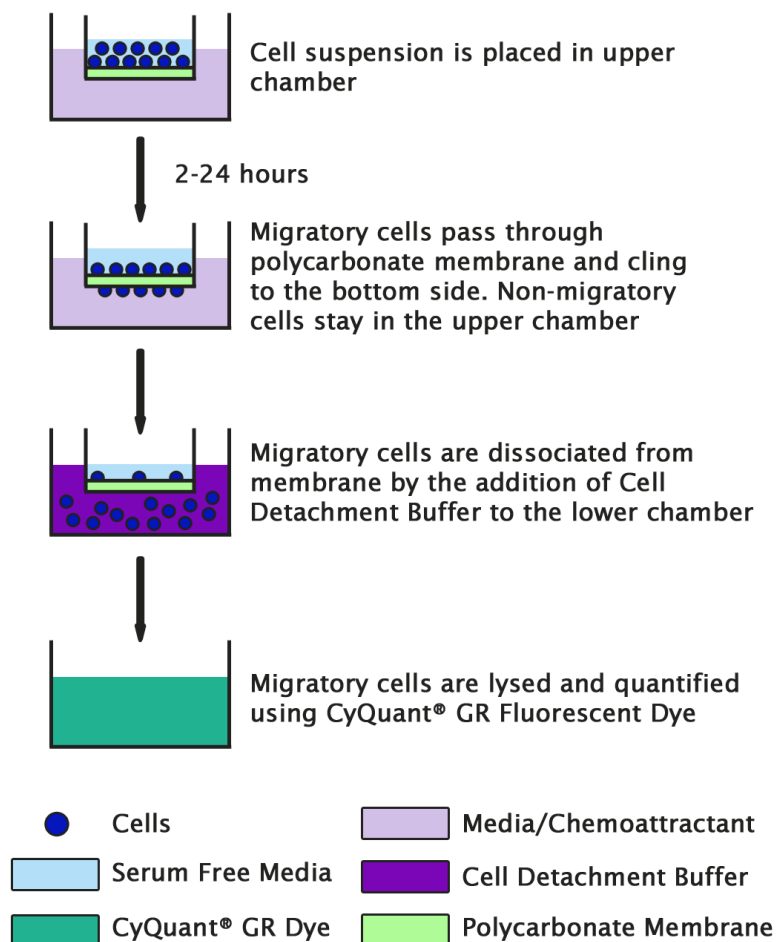
Cell migration is a highly integrated, multistep process that orchestrates embryonic morphogenesis, tissue repair and regeneration. It plays a pivotal role in the disease progression of cancer, mental retardation, atherosclerosis, and arthritis. The initial response of a cell to a migration-promoting agent is to polarize and extend protrusions in the direction of the attractant; these protrusions can consist of large, broad lamellipodia or spike-like filopodia. In either case, these protrusions are driven by actin polymerization and can be stabilized by extracellular matrix (ECM) adhesion or cell-cell interactions.

Cell Biolabs CytoSelect™ 96-well Cell Migration Assay Kit utilizes a polycarbonate membrane plate (5 µm pore size) to assay the migratory properties of cells. The kit does not require you to prelabel the cells with Calcein AM or remove non-migratory cells (i.e. cotton swabbing). Any migratory cells are first dissociated from the membrane, then lysed and detected with CyQuant® GR Dye.

Cell Biolabs CytoSelect™ 96-well Cell Migration Assay Kit provides a robust system for the quantitative determination of cell migration. The kit contains sufficient reagents for the evaluation of 96 samples. The 5 µm pore size is optimal for monocyte and macrophage cell migration. However, in the case of leukocyte chemotaxis, a smaller pore size (3 µm) is recommended.

The CytoSelect™ Cell Migration Assay Kit contains a polycarbonate membrane chamber (5 µm pore size) in a 96-well plate. The membrane serves as a barrier to discriminate migratory cells from non-migratory cells. Migratory cells are able to extend protrusions towards chemoattractants (via actin cytoskeleton reorganization) and ultimately pass through the pores of the polycarbonate membrane. These migratory cells are then dissociated from the membrane and subsequently detected with CyQuant® GR Dye.

Assay Principle



Related Products

1. CBA-100: CytoSelect™ 24-Well Cell Migration Assay (8µm, Colorimetric)
2. CBA-101: CytoSelect™ 24-Well Cell Migration Assay (8µm, Fluorometric)
3. CBA-102: CytoSelect™ 24-Well Cell Migration Assay (5µm, Fluorometric)
4. CBA-103: CytoSelect™ 24-Well Cell Migration Assay (3µm, Fluorometric)
5. CBA-104: CytoSelect™ 96-Well Cell Migration Assay (3µm, Fluorometric)
6. CBA-106: CytoSelect™ 96-Well Cell Migration Assay (8µm, Fluorometric)
7. CBA-111: CytoSelect™ 24-Well Cell Invasion Assay (Basement Membrane, Fluorometric)
8. CBA-120: CytoSelect™ 24-Well Wound Healing Assay (Light Microscopy)
9. CBA-125: Radius™ 24-Well Cell Migration Assay (Microscopy)
10. CBA-130: CytoSelect™ 96-Well Cell Transformation Assay (Soft Agar Colony Formation)

Kit Components

1. 96-well Cell Migration Plate (Part No. 10501): One sterile 96-well plate (see Figure 1 for components)
2. 96-well Cell Harvesting Tray (Part No. 10402): One 96-well tray
3. Cell Detachment Solution (Part No. 10403): One 20 mL bottle
4. 4X Lysis Buffer (Part No. 10404): One 10 mL bottle
5. CyQuant® GR Dye (Part No. 10105): One 75 µL tube

Materials Not Supplied

1. Migratory cell lines
2. Cell culture medium
3. Serum free medium, such as DMEM containing 0.5% BSA, 2 mM CaCl₂ and 2 mM MgCl₂
4. FBS or desired chemoattractant
5. Cell culture incubator (37°C, 5% CO₂ atmosphere)
6. Light microscope
7. 96-well plate suitable for a fluorescence plate reader
8. Fluorescence plate reader

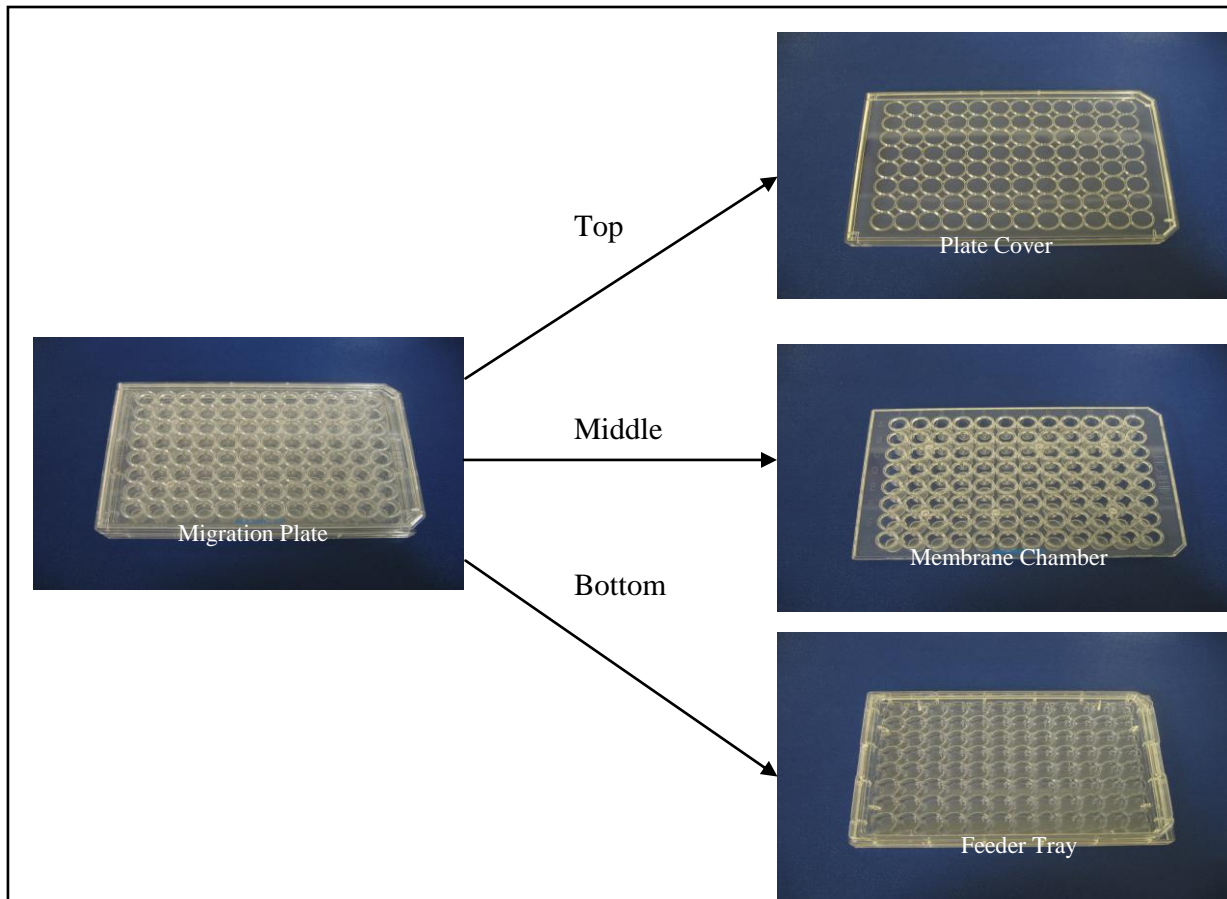


Figure 1: Components of the 96-well Cell Migration Plate.

Storage

Store all components at 4°C.

Assay Protocol

1. Allow the 96-well Migration Plate to warm up at room temperature for 10 minutes.
2. Prepare a cell suspension containing $0.5-5.0 \times 10^6$ cells/ml in serum free media. Agents that inhibit or stimulate cell migration can be added directly to the cell suspension.
(Note: Overnight starvation may be performed prior to running the assay)
3. Under sterile conditions, separate the cover and membrane chamber from the 96-well Migration Plate.
4. Add 150 μ L of media containing 10% fetal bovine serum or desired chemoattractant(s) to the wells of the feeder tray.

5. Place the membrane chamber back into the feeder tray (containing chemoattractant solution). **Ensure no bubbles are trapped under the membrane.**
6. Gently mix the cell suspension (without chemoattractant) from step 2 and add 100 μL to the membrane chamber.
7. Finally, cover the plate and transfer to a cell culture incubator for 2-24 hours.
8. Just prior to the end of the incubation, pipette 150 μL of prewarmed Cell Detachment Solution into wells of the clean, 96-Well Cell Harvesting Tray (provided).
9. Carefully remove the 96-well Migration Plate from the incubator. Separate the membrane chamber from the feeder tray.
Note: Retain the feeder tray for step 12.
10. Remove the cells/media from the top side of the membrane chamber by aspirating or inverting. Place the membrane chamber into the Cell Harvesting Tray containing 150 μL of Cell Detachment Solution (step 8). Incubate 30 minutes at 37°C.
11. Completely dislodge the cells from the underside of the membrane by gently tilting the membrane chamber several times in the Cell Detachment Solution.
12. In a clean 96-well plate (not provided), combine 75 μL of media from the feeder tray (step 9) with 75 μL of the detachment solution (step 11).
13. Prepare sufficient 4X Lysis Buffer/CyQuant® GR dye solution for all samples by diluting the dye 1:75 in 4X Lysis Buffer (for example, add 5 μL dye to 370 μL of 4X Lysis Buffer).
14. Add 50 μL of 4X Lysis Buffer/CyQuant® GR dye solution to each well (already containing 150 μL of Cell Detachment Solution). Incubate 20 minutes at room temperature.
15. Transfer 150 μL of the mixture to a 96-well plate suitable for fluorescence measurement. Read the fluorescence with a fluorescence plate reader at 480 nm/520 nm.

Example of Results

The following figures demonstrate typical with the CytoSelect™ Cell Migration Assay Kit. Fluorescence measurement was performed on SpectraMax Gemini XS Fluorometer (Molecular Devices) with a 485/538 nm filter set and 530 nm cutoff. One should use the data below for reference only. This data should not be used to interpret actual results.

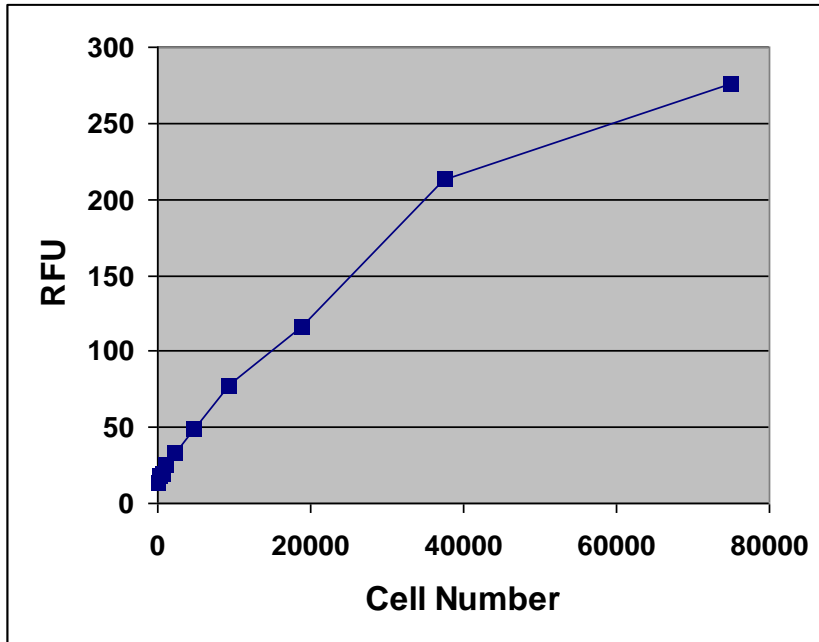


Figure 2: Quantitation of THP-1. THP-1 cells were titrated in Cell Detachment Buffer, then subsequently lysed and detected with 4X Lysis Buffer/Cyquant® GR Dye (150 µL cell suspension was mixed with 50 µL of 4X Lysis Buffer/dye).

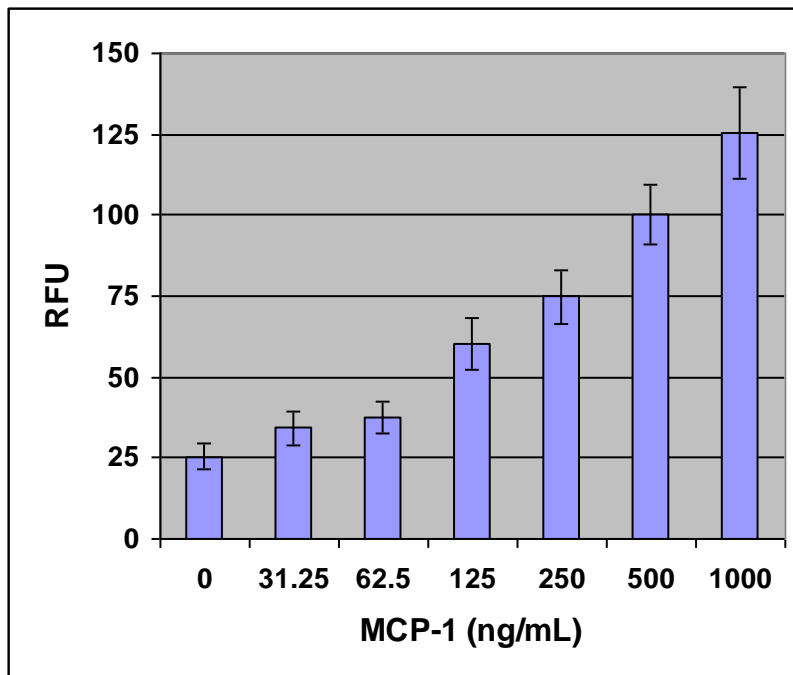


Figure 3. Human Monocytic THP-1 Chemotaxis. THP-1 cells were allowed to migrate toward MCP-1 for 2 hrs, 200,000 cells were used in each assay. Migratory cells were quantified by CyQuant® GR Dye as described in Assay Protocol.

References

1. Ridley AJ, Schwartz MA, Burridge K, Firtel RA, Ginsberg MH, Borisy G, Parsons JT, Horwitz AR. (2003) *Science* **302**, 1704-9.
2. Horwitz R, Webb D. (2003) *Curr Biol.* **13**, R756-9.
3. Lauffenburger DA, Horwitz AF. (1996) *Cell* **84**, 359-369.

Recent Product Citations

1. Aota, K. et al. (2020). Inhibition of JAK-STAT Signaling by Baricitinib Reduces Interferon- γ -Induced CXCL10 Production in Human Salivary Gland Ductal Cells. *Inflammation*. doi: 10.1007/s10753-020-01322-w.
2. Kumar, S. et al. (2020). Rationalized inhibition of mixed lineage kinase 3 and CD70 enhances life span and antitumor efficacy of CD8+ T cells. *J Immunother Cancer*. **8**(2):e000494. doi: 10.1136/jitc-2019-000494.
3. Cho, S.Y. et al. (2020). Amplification of transglutaminase 2 enhances tumor-promoting inflammation in gastric cancers. *Exp Mol Med*. doi: 10.1038/s12276-020-0444-7.
4. Van Cleemput, J. et al. (2020). An alphaherpesvirus exploits antimicrobial β -defensins to initiate respiratory tract infection. *J Virol*. pii: JVI.01676-19. doi: 10.1128/JVI.01676-19.
5. Elsayed, R. et al. (2020). Role of dendritic cell-mediated immune response in oral homeostasis: A new mechanism of osteonecrosis of the jaw. *FASEB J*. doi: 10.1096/fj.201901819RR.
6. O'Brien, J.J. et al. (2019). Inhibition of calcium-calmodulin-dependent phosphodiesterase (PDE1) suppresses inflammatory responses. *Mol Cell Neurosci*. doi: 10.1016/j.mcn.2019.103449.
7. Chung, K.W. et al. (2019). Mitochondrial Damage and Activation of the STING Pathway Lead to Renal Inflammation and Fibrosis. *Cell Metab*. pii: S1550-4131(19)30432-2. doi: 10.1016/j.cmet.2019.08.003.
8. Ouchi, R. et al. (2019). Modeling Steatohepatitis in Humans with Pluripotent Stem Cell-Derived Organoids. *Cell Metab*. pii: S1550-4131(19)30247-5. doi: 10.1016/j.cmet.2019.05.007.
9. Denard, B. et al. (2019). Regulating G protein-coupled receptors by topological inversion. *Elife*. **8**. pii: e40234. doi: 10.7554/eLife.40234.
10. Castro-Sánchez, S. et al. (2019). CX3CR1-deficient microglia shows impaired signalling of the transcription factor NRF2: Implications in tauopathies. *Redox Biol*. **22**:101118. doi: 10.1016/j.redox.2019.101118.
11. Betto, T. et al. (2019). Vascular endothelial growth factor receptor 1 tyrosine kinase signaling facilitates healing of DSS-induced colitis by accumulation of Tregs in ulcer area. *Biomed Pharmacother*. **111**:131-141. doi: 10.1016/j.biopha.2018.12.021.
12. Llaudo, I. et al. (2019). C5aR1 regulates migration of suppressive myeloid cells required for costimulatory blockade-induced murine allograft survival. *Am J Transplant*. **19**(3):633-645. doi: 10.1111/ajt.15072.
13. Kovacsovics, T.J. et al. (2018). Combination of the low anticoagulant heparin CX-01 with chemotherapy for the treatment of acute myeloid leukemia. *Blood Adv*. **2**(4):381-389. doi: 10.1182/bloodadvances.2017013391.
14. Hakuno, D. et al. (2018). Hepatokine α 1-Microglobulin Signaling Exacerbates Inflammation and Disturbs Fibrotic Repair in Mouse Myocardial Infarction. *Sci Rep*. **8**(1):16749. doi: 10.1038/s41598-018-35194-w.
15. Owens III, A.P. et al. (2018). Tefillin use induces remote ischemic preconditioning pathways in healthy men. *Am J Physiol Heart Circ Physiol*. **315**(6):H1748-H1758. doi: 10.1152/ajpheart.00347.2018.

16. Mohamed, S.I.A. et al. (2018). Dendritic cells pulsed with generated tumor cell lysate from *Phyllanthus amarus* Schum. & Thonn. induces anti-tumor immune response. *BMC Complement Altern Med.* **18**(1):232. doi: 10.1186/s12906-018-2296-4.
17. Yamada, S. et al. (2018). Inhibition of Local Macrophage Growth Ameliorates Focal Inflammation and Suppresses Atherosclerosis. *Arterioscler Thromb Vasc Biol.* **38**(5):994-1006. doi: 10.1161/ATVBAHA.117.310320.
18. Hernández-Ruiz, E. et al. (2018). The Polycomb proteins RING1B and EZH2 repress the tumoral pro-inflammatory function in metastasizing primary cutaneous squamous cell carcinoma. *Carcinogenesis.* **39**(3):503-513. doi: 10.1093/carcin/bgy016.
19. Kitadate A, et al. (2018). Histone deacetylase inhibitors downregulate CCR4 expression and decrease mogamulizumab efficacy in CCR4-positive mature T-cell lymphomas. *Haematologica.* **103**(1):126-135. doi: 10.3324/haematol.2017.177279.
20. Stabler, T.V. et al. (2017). Chondroitin Sulfate Inhibits Monocyte Chemoattractant Protein-1 Release From 3T3-L1 Adipocytes: A New Treatment Opportunity for Obesity-Related Inflammation? *Biomark Insights.* **12**:1177271917726964. doi: 10.1177/1177271917726964
21. Aota, K. et al. (2017). Cepharanthine Inhibits IFN- γ -Induced CXCL10 by Suppressing the JAK2/STAT1 Signal Pathway in Human Salivary Gland Ductal Cells. *Inflammation.* doi: 10.1007/s10753-017-0662-x
22. Luo, X. et al. (2017). Methylation-mediated loss of SFRP2 enhances melanoma cell invasion via Wnt signaling. *Am J Transl Res.* **8**(3):1502-9.
23. Shikhagaie, M.M. et al. (2017). Neuropilin-1 Is Expressed on Lymphoid Tissue Residing LTi-like Group 3 Innate Lymphoid Cells and Associated with Ectopic Lymphoid Aggregates. *Cell Rep.* **18**(7):1761-1773. doi: 10.1016/j.celrep.2017.01.063.
24. Vazquez Rodriguez, G. et al. (2017). Estradiol Promotes Breast Cancer Cell Migration via Recruitment and Activation of Neutrophils. *Cancer Immunol Res.* **5**(3):234-247. doi: 10.1158/2326-6066.CIR-16-0150.
25. Cao, Q. et al. (2016). Myeloid deletion of α 1AMPK exacerbates atherosclerosis in LDL receptor knockout (LDLRKO) mice. *Diabetes.* **65**:1565-1576.
26. Yao, L. et al. (2016). Characterization of liver monocytic myeloid-derived suppressor cells and their role in a murine model of non-alcoholic fatty liver disease. *PLoS One.* **11**:e0149948.
27. Jiang, W. et al. (2016). Infiltration of CCR2+ Ly6Chigh proinflammatory monocytes and neutrophils into the central nervous system is modulated by nicotinic acetylcholine receptors in a model of multiple sclerosis. *J Immunol.* **196**:2095-2108.
28. Hargarten, J. C. et al. (2015). *Candida albicans* quorum sensing molecules stimulate mouse macrophage migration. *Infect Immun.* **83**:3857-3864.
29. Kondo, Y. et al. (2015). Differential expression of CX3CL1 in hepatitis B virus-replicating hepatoma cells can affect the migration activity of CX3CR1+ immune cells. *J Virol.* **89**:7016-27.
30. Baek, J. H. et al. (2015). IL-34 mediates acute kidney injury and worsens subsequent chronic kidney disease. *J Clin Invest.* doi: 10.1172/JCI81166.

License Information

This product is provided under an intellectual property license from Life Technologies Corporation. The purchase of this product conveys to the buyer the non-transferable right to use the purchased product and components of the product only in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The sale of this product is expressly conditioned on the buyer not using the product or its components, or any materials made using the product or its components, in any

activity to generate revenue, which may include, but is not limited to use of the product or its components: (i) in manufacturing; (ii) to provide a service, information, or data in return for payment; (iii) for therapeutic, diagnostic or prophylactic purposes; or (iv) for resale, regardless of whether they are resold for use in research. For information on purchasing a license to this product for purposes other than as described above, contact Life Technologies Corporation, 5791 Van Allen Way, Carlsbad CA 92008 USA or outlicensing@lifetech.com.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

©2004-2020: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.