

Cell Secretion Kit

Antibody-Specific

Catalog Numbers

Human specific: 20040

Mouse specific: 20050

Store at 4 °C and -20 °C.

MKTG-001-R00
(PN 0006303 on BOM)
October 6, 2010

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Companion Documents

- Celigo Cytometer User Guide
- Celigo Cell Secretion Application Guide
- LEAP Cell Processing Workstation User Manual
- LEAP Cell Secretion Application Guide

Storage, Handling, and Stability

Initially, store Components A and C at -20 °C. Store the rest of the kit at 4 °C. Shield Antibody Detection Reagent (B) and Cell Detection Reagent (C) solutions, as well as stained plates, from light.

Upon reconstitution, follow specific storage conditions for each reagent.

The components of this system are stable for six months when stored under the recommended conditions.

Warning

For research use only and not for resale. Not recommended or intended for diagnosis or treatment of disease in humans or animals.

1. Introduction to Cell Secretion Kit

The Cell Secretion Kit is designed to enable the rapid quantitative analysis of cells secreting proteins using the Celigo cytometer and LEAP cell processing workstation. Analysis of cells based on protein secretion levels (e.g., antibody, growth factors, cytokines) is a particular challenge because the secreted product does not remain associated with the cell. The Cell Secretion Kit provides reagents for the *in situ* capture, detection, and measurement of secreted antibodies from single cells, allowing the user to profile secretion characteristics of populations at the single-cell level.

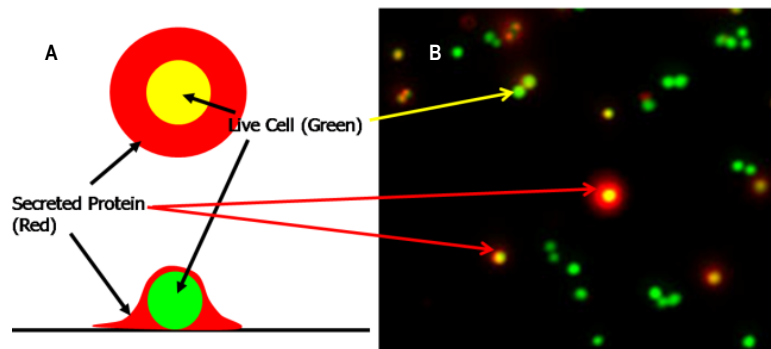


Fig. 1. Schematic and Photomicrograph of Cell Secretion Assay. (A) Schematic illustrating both top and side view of labeled cell and secreted protein. The cells are labeled with a green fluorescent dye that can appear yellow due to mixing of red and green colors. The capture reagent traps the secreted antibody around the immediate vicinity of the cell. (B) Photomicrograph of cells labeled with a cytoplasm stain (green) and a stain specific for human antibodies (red). The red area around the cells represents the captured secreted antibody.

The Cell Secretion Kit can be used with the Celigo cytometer and LEAP workstation to image and quantify secretion. Additionally, the LEAP workstation provides a means to clone or enrich cells with laser-mediated elimination of poorly secreting cells, leading to clones of highly secreting cells.

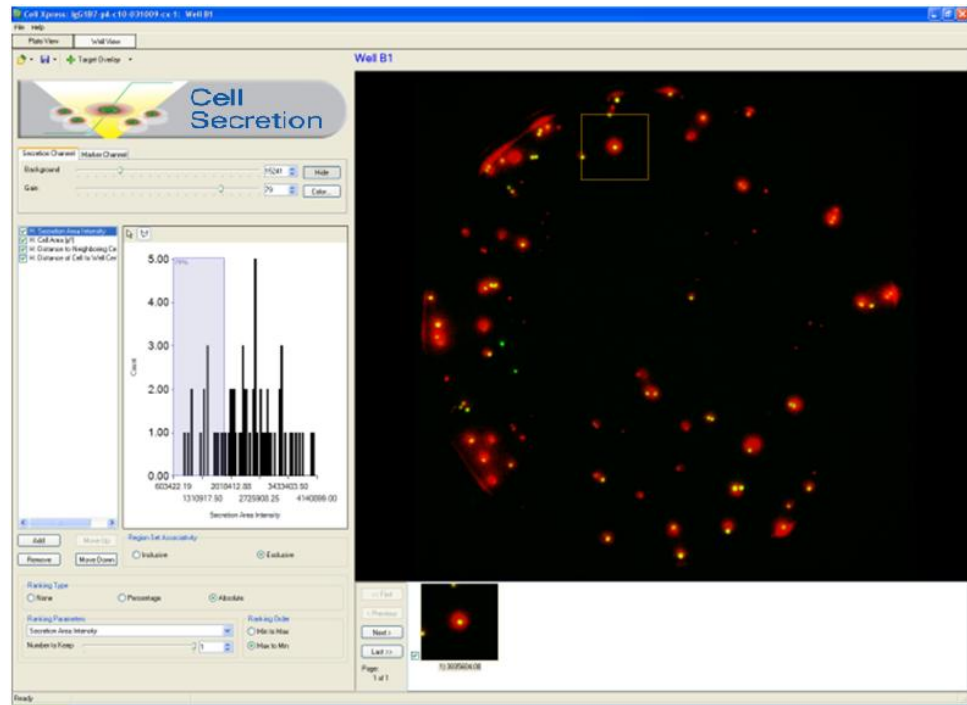


Fig. 2. Whole-Well View of Cell Population Secreting Mouse Antibodies. Highest secreting cell outlined as well as displayed below whole well view.

Rather than isolate clones (via limiting dilution cloning) and then searching through all the clones for the best secretor, the Cell Secretion Application on the LEAP workstation ranks the secretors and then allows the user to decide which cells to keep. The Cell Secretion Application (Hanania et. al., 2005) is executed on the LEAP workstation, which integrates high-throughput live cell imaging and in situ laser-mediated cell manipulation (Koller et al., 2004).

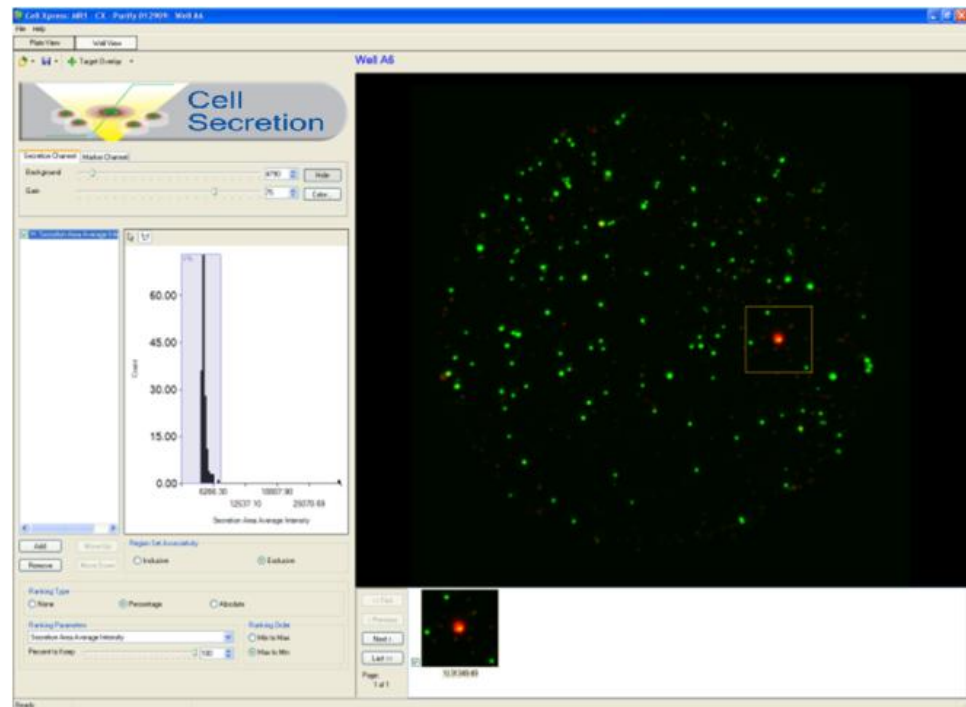


Fig. 3. Whole-Well View of Cell Population Exhibiting Secretion of Antibodies from Only One Cell. Assay exhibits sufficient sensitivity to detect secretion from only one cell within a large population. Using the LEAP workstation, rare cells within a population can be purified/cloned using laser elimination of undesired cells.

The Cell Secretion Kit includes a multi-step process that includes: (1) *In situ* capture and labeling of secreted protein product (Fig. 1); (2) high-throughput fluorescent imaging of the well; and (3) image analysis for identification, classification, and analysis of each individual secreting cell (Fig. 2, 3).

2. Overview of Cell Secretion Kit

The Cell Secretion Kit provides validated reagents, plates, and biological protocols to visualize and analyze the amount of antibody secreted from individual cells. This kit provides a live cell assay for secreted antibody, allowing the cloning of cells (using a LEAP workstation). Supplied with the Cell Secretion Kit are reagents for capturing and labeling the secreted antibody. This capture and labeling occurs proximal to each cell and thus allows associating a measured secretion with a particular cell using image analysis. Analysis can be performed on either a Celigo cytometer or LEAP workstation. The LEAP workstation can be used to perform secretion cloning, enabling the cloning/enrichment of the secreting cells for large scale production of antibodies (purification reagents included). For a detailed description of the complete process, see the respective application guides.

3. Materials Supplied

- Antibody Capture Reagent (Tube A) (store at -20 °C)
- Antibody Detection Reagent (Tube B) (store at 4 °C, DO NOT FREEZE)
- Cell Detection Reagent (Tube C) (store at -20 °C)
- Antibody Detection Reagent Buffer (Tube D) (store at 4 °C)
- 20x PhotoTherm Reagent (2 Tube E) (store at 4 °C)
- Cyntellect C-lect 384-Well Plates (2 plates) (store at RT)

4. Materials Required But Not Supplied

- DMSO (Anhydrous)
- Sterile Vials (for storing aliquots of reagents)

5. Master Stock Solutions



NOTE: All handling should be done under sterile conditions with sterile containers and pipetting devices.

- **Antibody Capture Reagent Stock (Tube A)**

The Antibody Capture Reagent (15 µl, tube A) is stable for at least 6 months when kept at -20 °C. After the first thaw, it is then stable for at least 3 months at 4 °C. Avoid repeated freeze/thaw.
- **Antibody Detection Reagent Stock (Tube B)**
 1. Add 1.0 ml of Antibody Detection Reagent Buffer (tube D) to the Antibody Detection Reagent Stock (tube B).
 2. Mix by gentle pipetting. Aliquot in 200 µl quantities and store at 4 °C. (Solution is stable for 3 months, DO NOT FREEZE).
- **Cell Detection Reagent Stock (Tube C)**
 1. Add 54 µl of anhydrous DMSO to the Cell Detection Reagent Stock (tube C).
 2. Mix by gentle pipetting. Aliquot in 10 µl quantities and store at -20 °C. (Solution is stable frozen for 6 months. Avoid repeated freeze/thaw).

6. Protocol

6.1 Cell Pre-Labeling

When using the Cell Secretion Assay, pre-label cells with the Cell Detection Reagent.

1. Add 1 μ l Cell Detection Reagent Stock (tube C) per 1.0 ml of cell suspension containing $5-10 \times 10^5$ cells/ml in serum-free (preferred) or regular medium. This corresponds to a 1/1000 dilution.
2. Incubate for 30-60 minutes in a 37 °C incubator, protected from light.
3. Wash cells once in serum-free medium using centrifugation.
4. Count pre-labeled cells concentration using a hemacytometer.

6.2 Preparing Cell & Reagent Mix

Adjust the volume of Cell & Reagent Mix based on the number of wells used on a 384-well C-lect plate. It is recommended to use 100-300 cells/well in a volume of 20 μ l/well. Too high a cell density may lead to overlapping secretion areas between cells.

Prepare the Cell & Reagent Mix *immediately before* plating the cells.



NOTE: Do not use serum (e.g. FBS) in the medium used to incubate the cells. The use of serum in the culture medium may cause the detection reagent to aggregate and result in non-specific staining.

For a full 384-well plate, prepare the following mix:

1. Dilute the cells pre-labeled with the Cell Detection Reagent to a concentration of 5,000-15,000 cells/ml in serum-free media by adding 50,000-150,000 cells to a total volume of 10 ml. This corresponds to 100-300 cells per 20 μ l well volume.
2. Add 5 μ l of Antibody Capture Reagent (tube A). This corresponds to a 1/2000 dilution.
3. Add 400 μ l of Antibody Detection Reagent (tube B). This corresponds to a 1/25 dilution.
4. Mix gently.
5. Dispense 20 μ l of Cell & Reagent Mix into each well of the provided 384-well C-lect plate.
6. Centrifuge the plate at 100 g (or ~500 rpm in most centrifuges) for 30 sec in order to settle the cells at the bottom of the wells.
7. Incubate the plate overnight in the 37 °C incubator.

6.3 Reading the Assay

Prior to reading the assay on either platforms, add PhotoTherm reagent to the wells. PhotoTherm reagent is required to eliminate the cells by laser targeting on the LEAP workstation using the Cell Secretion application. For further information, refer to the Celigo and LEAP application guides.



NOTE: Avoid disturbing the cells on the bottom of the plate. For all liquid additions, it is highly recommended that you use a multichannel motorized pipettor or a robot, generally at the lowest aspiration and delivery rates (2-6 μ l/sec). A Thermo Scientific Matrix Equalizer Pipette can be used for dispensing fluids. Ensure that the tip of the pipettor does not get too close to cells.

1. Prepare a 2x solution of PhotoTherm by adding 1 ml of the stock (tube E) to 9 ml of serum-free medium. This corresponds to a 1/10 dilution.
2. Warm the solution to 37 °C in water bath or incubator.
3. Gently dispense 20 μ l of the 2x solution of PhotoTherm in each well of the 384-well plate. Ensure that the pipettor's tips touch the liquid interface before starting dispensing the PhotoTherm so that the cells at the bottom of the well are not being displaced.
4. Acquire the images on a Celigo cytometer or process the plate on a LEAP workstation.

6.4 Washes After Processing on the LEAP Workstation

After the plate has been imaged and processed on the LEAP workstation, each well must be washed from the Cell Secretion reagents in order to allow the remaining cells to grow and clones to expand.

1. Carefully remove 20 μ l from each well and add 20 μ l of fresh pre-warmed serum-free media (alternatively, you can wash with HBSS or PBS).
2. Incubate at room temperature for 1-2 minutes.
3. Repeat washing 3-5 times (steps 1 and 2).
4. Add 20 μ l of cell culture media containing appropriate concentration of serum and return the plate to the 37 °C incubator.

7. Quick Reagent Concentration Guide

Reagent	Recommended Final Dilution
Cell Detection Reagent (Tube C)	1/1000
Antibody Capture Reagent (Tube A)	1/2000
Antibody Detection Reagent (Tube B)	1/25
PhotoTherm (Tube E)	1/20

8. Fluorescent Dye Detection

All live cells are fluorescently stained green (Ex 490nm/ Em 515nm) and secreted protein is fluorescently stained red (Ex 480-550 nm / Em 575-629 nm).

Appendix A Assay Data

A.1 Quantification of Antibody Secretion with Cell Secretion

Cell clones expressing antibody were analyzed by both the Cell Secretion Kit and HPLC. The HPLC assay analyzed supernatant from 10^6 cells incubated for 24 hours in 1 ml using a protein A column calibrated against purified antibody product. Results (Figs. A1 – A2) indicate good linear response of the assay.

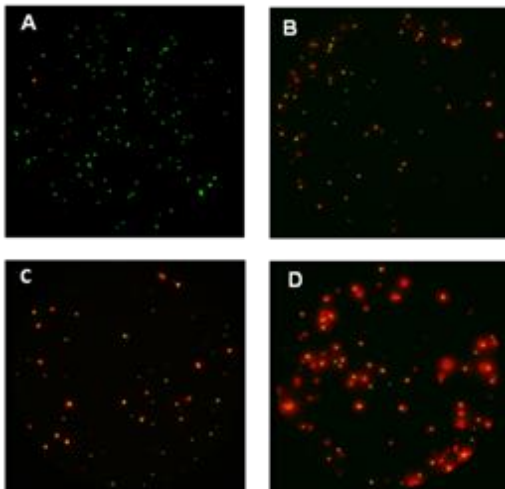


Fig. A1. Images of Cell Secretion Assay. Illustrated are (A) non-secreting cells and (B – D) cells with increasing secretion rates (see Fig. A2).

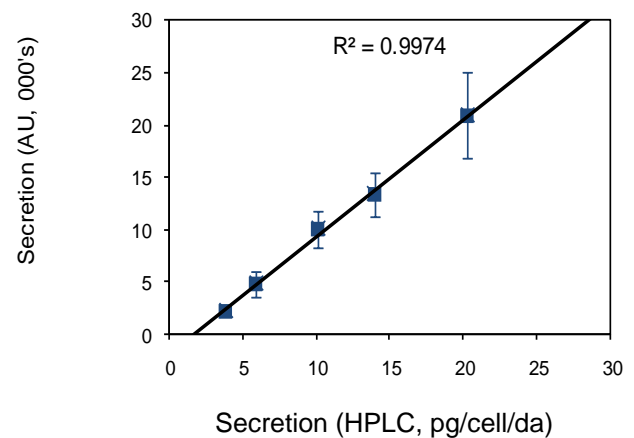


Fig. A2. Quantitative Relationship between Cell Secretion Data and Conventional HPLC Assay.

Appendix B Performance Data

The *in situ* secretion assay exhibited excellent reproducibility (Fig. B1). This assay included 45 wells of cells with a relatively low secretion rate (5 pg/cell/day) and 45 wells of cells with a high secretion rate (20 pg/cell/day). The well-to-well variability for both levels of secretion was ~8%.

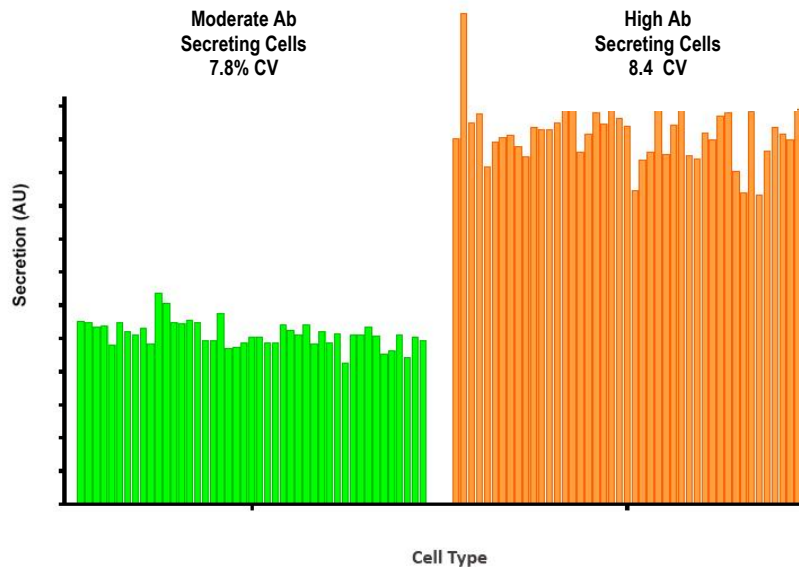


Fig. B1. Performance Data for Cell Secretion for Cells Producing Humanized Antibodies.

References

- Hanania, E.G, A. Fieck, J. Stevens, L.J. Bodzin, B.O. Palsson, M.R. Koller. 2005. Automated In situ Measurement of Cell-Specific Antibody Secretion and Laser-Mediated Purification for Rapid Cloning of Highly-Secreting Producers. *Biotechnology and Bioengineering*, 91(7):872-876, 2005.
- Koller, M.R., E.G. Hanania, J Stevens, T.M. Einfeld, G.C. Sasaki, A. Fieck, B.O. Palsson. 2004. High-Throughput Laser-Mediated In situ Cell Purification With High Purity and Yield. *Cytometry* 61A:153-161.

Cyntellect Cell Secretion Kit

Published By	<p>Cyntellect®, Inc. 6620 Mesa Ridge Road San Diego, CA 92121 Web Site: www.cyntellect.com</p>
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Appropriate Use	<p>For research and development uses, only. Not for use in diagnostic or therapeutic procedures. The LEAP workstation is designed to perform both user-interactive and automated for the measurement, analysis, and induction of events in cell specimens by imaging cells in microplates, analyzing images, and inducing changes in selected cells via laser irradiation. The LEAP workstation is designed to be used in a temperature and humidity controlled laboratory environment that is free from aerosols, liquid sprays or spills, and excessive airborne dust.</p> <p>Use of the LEAP workstation and software is governed by the <i>LEAP Terms and Conditions</i> and the <i>Cyntellect Inc. Software End User License Agreement</i>. For questions, please contact Cyntellect Customer Service at 877.584.9150.</p>