The use of a fluorescence cell stain provides a more reliable and conclusive method for determining CHO CellTracker Green CMDFA. The CloneSelect Imager FL software automatically analyzed the fluorescence images to provide consistent determination of cell confluence and cell number.

Automated generation of growth curves. Regulations for cell line development are increasingly more stringent. Providing evidence that a Colony forming assays. The Single cell identification with the aid of fluorescence is more conclusive and robust, helping to meet stringent regulatory demands in cell line development.

METHODS

CHO-S CELL STAINING WITH CELL TRACKER GREEN

- CellTracker Green CMDFA (Life Technologies), a cell membrane permeable dye used to monitor cell location, was resuspended in DMSO to a concentration of 10mM and then diluted to 10µM in XP Media CHO Growth A (Part# #0860, Molecular Devices) supplemented with 4mM L-glutamine.
- CHO-S cells were initially cultured in XP Media CHO Growth A supplemented with 4mM L-glutamine and then stained with 10µM of CellTracker Green and incubated for 30 min at 37°C. After incubation, CHO-S cells were washed twice with XP Media CHO Growth A supplemented with 4mM L-glutamine.
- The stained CHO-S cells were seeded into a 96-well plate at a density of ~1 cell/well for all wells except well B2. Well B2 was seeded at a density of ~300 cells/well to facilitate setting the focus on the CloneSelect Imager FL.

WHITE LIGHT AND FLUORESCENCE IMAGING OF CELLTRACKER GREEN STAINED CHO-S CELLS

- The 96-well plates were imaged both in white light and fluorescence on the CloneSelect Imager FL. A FITC filter set was used for fluorescence imaging.

CONCLUSIONS

- The use of a fluorescence cell stain provides a more reliable and conclusive method for determining monoclonality than the traditional white light method.
- The CloneSelect Imager FL and CellTracker Green staining can be used to fluorescently detect cells and determine cell number in a well for monoclonality verification. This method can be used for other fluorescence stains as well.
- The CloneSelect Imager FL provides fast, high quality white light and fluorescence imaging. The CloneSelect Imager FL software automatically performs cell count analysis on the fluorescence images making it easy to identify wells that contain a single cell.