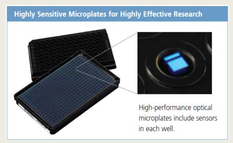
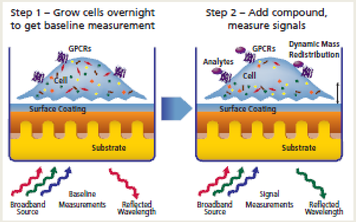
Non-Invasive Label-Free Studies of Receptor Activation in Lonza® Primary & Mesenchymal Stem Cells Using the EnSpire® Multimode Plate Reader & JANUS® Automated Workstation

Heidi Morgan, M.S., Vincent Dupriez, Ph.D., Tim Cloutier, Ph.D., PerkinElmer, Inc. Waltham, MA USA
Kristin Atze, Leon de Bruin, Lonza Cologne GmBH, Koeln, Germany

1 Abstract

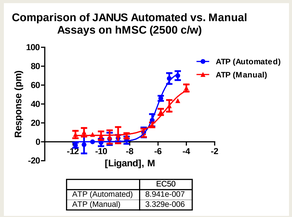
There is growing demand for more physiologically-relevant assay platforms, leading to increased adoption of both label-free technologies and stem cells in research. Automated systems are also needed to facilitate research by increasing cell-based assay efficiency, reproducibility, and performance. To address these needs, methods utilizing the PerkinElmer EnSpire™ Multimode Plate Reader with Corning® Epic® label-free technology and the JANUS® Automated Workstation have been developed to non-invasively identify and characterize multiple Ion Channel and GPCR activation in Lonza® Cloneticstm Human Umbilical Vein Endothelial Cells (HUVEC) and Poietics™ human Mesenchymal Stem Cells (hMSC). By monitoring the ligand-induced dynamic mass redistribution (DMR) in living cells with no need for cellular or ligand modification, the EnSpire label-free platform detects cellular responses from endogenously expressed receptors and ion channels. This obviates the need to engineer cells to over-express receptors of interest, thus greatly reducing the possibility of altering cellular biology. These data demonstrate the applicability of label-free technology for primary and stem cell research and the utility of liquid handling automation to ensure highly reproducible data from these primary cell assays.



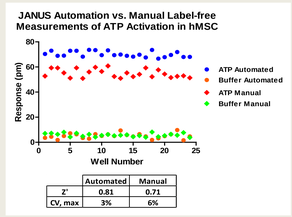


Cell-Based Assays
Epic® technology measures changes in light refraction resulting from dynamic mass redistribution (DMR) within the cell which occurs in response to receptor activation or deactivation in a zone within the cell's monolayer. The change is indicated by a change in wavelength.

2 Automating Stem Cell Assays



	EC50
ATP (Automated)	8.941e-007
ATP (Manual)	3.329e-006



	Automated	Manual
Z'	0.81	0.71
CV, max	3%	6%

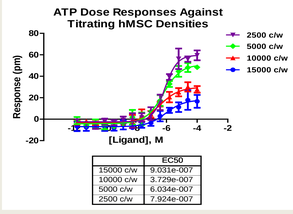
All liquid handling steps were performed both manually and on the JANUS Automated Workstation with comparable results.

- The advantage of automating the label-free steps is to provide a robust and reliable easy-to-use protocol resulting in significant time savings and enhanced reproducibility.

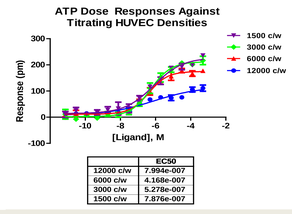
3 Robust Label-free Detection

	EC50
132N1-rP2Y11 (10000 c/w)	7.469e-007
hMSC (2500 c/w)	7.923e-007
HUVEC (3000 c/w)	5.949e-007

Three cell types were used to test the response of either recombinantly or endogenously expressed Purinergic receptors to ATP: Human astrocytoma cells (1321N1), human mesenchymal stem cells (hMSC), and human umbilical vein endothelial cells (HUVEC). A robust 50-fold window (pm) is observed with the endogenously expressed receptors, demonstrating the sensitivity of the EnSpire with label-free platform.



	EC50
15000 c/w	9.031e-007
10000 c/w	3.729e-007
5000 c/w	6.034e-007
2500 c/w	7.924e-007

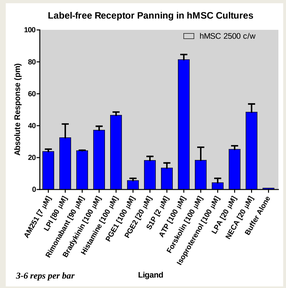


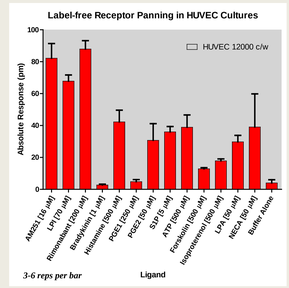
	EC50
12000 c/w	7.994e-007
6000 c/w	4.168e-007
3000 c/w	5.278e-007
1500 c/w	7.876e-007

Cell density titrations of hMSC or HUVEC vs. ATP performed to identify the optimal assay signal and window for study.

- Lower numbers of cells per well increases the assay window by ~ 3-fold relative to higher densities, thus providing an additional cost savings benefit when running this simple assay workflow.

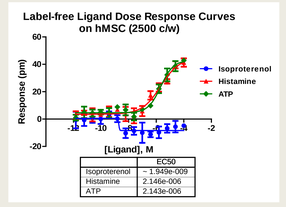
4 Label-free GPCR Receptor Panning



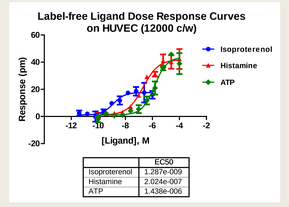


The label-free platform robustly monitors the activation of GPCRs coupled to various pathways within multiple cell types.

- The absolute response (pm) +/- SD of mean with various GPCR activators and several negative control compounds.
- AM251, LPI and rimonabant are three known agonists of the GPR55 cannabinoid receptor, which is known to couple only to $G\alpha_{12/13}$ proteins.



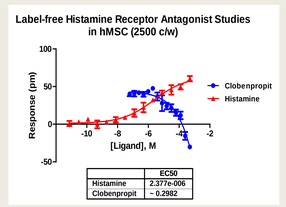
	EC50
Isoproterenol	~ 1.949e-009
Histamine	2.146e-006
ATP	2.143e-006



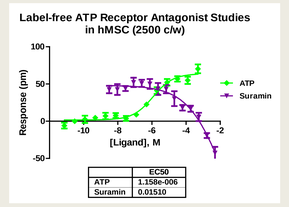
	EC50
Isoproterenol	1.287e-009
Histamine	2.024e-007
ATP	1.438e-006

Representative DRCs for several agonists in both cell types:

- Histamine and ATP act in a similar manner against the endogenous Histamine and Purinergic receptors in both hMSC and HUVEC (positive DMR response).
- Isoproterenol has a differential effect in hMSC relative to the HUVEC system (negative DMR response).
- This points to potentially shared (ATP, Histamine) or divergent (isoproterenol) couplings and/or divergent cellular response to a common coupling for the receptors of these ligands.



	EC50
Histamine	2.377e-006
Clobenpropit	~ 0.2982

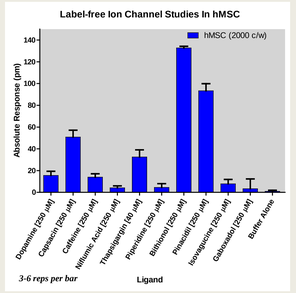


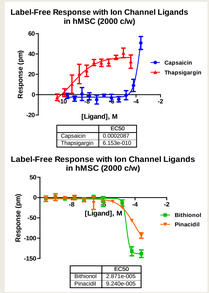
	EC50
ATP	1.158e-006
Suramin	0.01510

Testing the specificity of the agonist response using antagonists in the hMSC Purinergic and Histamine pathways.

- Suramin and Clobenpropit clearly inhibit ATP and Histamine agonist activity, respectively.
- This inhibition confirms that Purinergic and Histamine receptor activation can be robustly measured using the EnSpire label-free cellular assay.

5 Label-free Ion Channel Receptor Panning





	EC50
Capsaicin	0.0002097
Thapsigargin	6.153e-010

	EC50
Bithionol	2.871e-005
Pinacidil	9.240e-005

Label-free robustly monitors the activation of different ion channels in hMSCs.

- The absolute response (pm) +/- SD of mean with various ion channel activators and several negative control compounds.
- Capsaicin (a known activator of the TRPV1 vanilloid/capsaicin receptor, potentially activating another type of ion channel at the high concentration used here) and Thapsigargin (potent inhibitor of sarco-endoplasmic reticulum Ca^{2+}-ATPases), provide large positive pm responses in hMSC.
- Bithionol (an activator of calcium-activated potassium channels) and Pinacidil (an activator of ATP-sensitive potassium channels) provide large negative pm responses.

6 The EnSpire Label-free Platform Offers:

- A versatile tool for pathway-independent, global analysis of basic systems biological research and physiologically-relevant drug discovery.
- A non-invasive tool to detect GPCR and Ion channel activation in stem and primary cell types.
  - o Suggesting it is a viable platform for studying embryonic stem cells and derived cell types.
- In conjunction with the JANUS AWS, significant reduced cost, assay development time and reagents requirements for primary and stem cell research.
- In collaboration with the high quality and fully optimized Lonza products, a sensitive and robust system that easily detects both endogenously and recombinantly expressed receptors in receptor panning experiments.