nanoString

nCounter® Stem Cell Characterization Panel

Cellular Therapy • Regenerative Medicine • Manufacturing

Deeply characterize and optimize your stem cell development with the nCounter Stem Cell Characterization Panel. This panel measures the eight essential components of stem cell biology. Evaluate viability, confirm functionality and determine pluripotency with a single robust, automated, and reproducible assay. Assess stem cell health during production and easily detect contamination to quickly optimize cell culture conditions and expedite your research.



Product Highlights

- Directly profile 770 genes involved in stem cell biology:
 - Stemness

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- Pluripotency
 - Regulatory Signaling Differentiation Signaling
- Epigenetics
- Lineage Specification

Mechano-Signaling

Metabolism

- Characterize stem cells during development
 - Confirm pluripotency
 - Understand reprogramming failures
 - Assess activation/differentiation status
- Screen for viability
- Confirm expanded cell function via cell signaling molecules and pathway activation
- Confirm cell viability after gene editing
- Detect mycoplasma contamination
- Generate data in 24 hours with less than 30 minutes hands on time and simple data analysis

Feature	Specifications
Number of Targets	770 (Human and Mouse), including 12 internal reference genes
Sample Input - Standard (No amplification required)	25-300 ng
Sample Input - Low Input	As little as 1 ng with nCounter Low Input Kit and Primer Pools (sold separately)
Sample Type(s)	Cultured cells/cell lysates, sorted cells, FFPE-derived RNA, total RNA, fragmented RNA
Customizable	Add up to 55 unique genes with Panel-Plus or up to 10 custom protein targets
Time to Results	Approximately 24 hours
Data Analysis	nSolver™ Analysis Software, ROSALIND®

FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.

The Stem Cell Therapy Workflow

Culturing stem cells is a delicate art. The environment needs to be tightly controlled and the cells need to be checked at each stage to ensure that they are differentiating as desired. The Stem Cell Characterization Panel can be used throughout development processes to confidently characterize stems cells and understand pluripotency.



Functional Annotations

The Stem Cell Characterization Panel provides a comprehensive view of a stem cell's life cycle. Probes cover all eight essential components of stem cell biology, as outlined in the table below.

The Eight Essential Components of Stem Cell Biology

Stemness	Pluripotency	Regulatory Signaling	Epigenetics	Mechano- Signaling	Metabolism	Differentiation Signaling	Lineage Specification
 Stem Cell Self Renewal Stem Cell Proliferation Cell Cycle Senescence/ Quiescence Autophagy Apoptosis Anti-Apoptosis 	 PSC Pluripotency Markers and Regulators Naive State/ Primed State 	Regulatory Pathways • Wnt/B-catenin Pathway • Hedgehog Signaling • AP-1 Signaling • PI3K-AKT- mTOR Pathway • MAPK Pathway • JAK/STAT Pathway • Notch Signaling	Epigenetic Mechanisms • DNA Methylation • Histone Acetylation & Methylation	 Mechano- Signaling Rho/ROCK Signaling integrin/Cad herin Signaling Hippo pathway 	 Metabolism Oxidative Stress Response Hypoxia Response Amino Acid Metabolism Fatty Acid Metabolism Glutamine Metabolism Glucose Metabolism 	 Differentiation Signaling and Pathways TGFB Signaling Cytoskeletal Reorganization MET/EMT Signaling HOX Gene Activation 	 Differentiation Lineages Endodermal/ Ectodermal/ Mesodermal Lineage Markers Key somatic cell types

Contamination Detection

Mycoplasma is a common contaminant in cultured cells. Mycoplasma compete with stem cells for nutrients and can have a profound impact on global gene expression levels within the cells. The Stem Cell Characterization Panel contains a probe to detect mycoplasma, allowing for quick and easy detection of culture contamination. The panel can also be customized by adding up to 55 genes of your choice with a Panel Plus spike-in for studying additional sources of potential contamination.

Customization with Panel Plus

Customize your research project by adding up to 55 user-defined genes of interest with nCounter[®] Panel Plus. Panel Plus capacity enables researchers to address areas like specific lineage interests, such as cardiomyocytes, neurons, retinal cells and beta cells.

nSolver[™] Analysis Software

NanoString offers advanced software tools that address the continuous demands of data analysis and the need to get simple answers to specific biological questions easily. Genes included in the Stem Cell Characterization Panel are annotated to allow for efficient analysis of relevant pathways.

Analysis Modules available for Stem Cell Characterization:

- Normalization
- Gene Set Analysis
- Individual
 Pathway Analysis

Quality Control

• Built-in compatibility for Panel-Plus and Protein analysis

Differential Expression

ROSALIND®

ROSALIND is a cloud-based platform that enables scientists to analyze and interpret differential gene expression data without the need for bioinformatics or programming skills. ROSALIND makes analysis of nCounter data easy, with guided modules for:

- Normalization
- Quality Control
- Individual Pathway Analysis
- Differential Expression
- Gene Set Analysis

nCounter customers can access ROSALIND free of charge at https://www.rosalind.bio/nanostring.



Ordering Information

Product	Product Description	Quantity	Catalog Number
nCounter Human Stem Cell Characterization Panel	770 genes, including 12 internal reference genes for data normalization	12 Reactions	XT-CSO-HSCC-12
nCounter Mouse Stem Cell Characterization Panel	770 genes, including 12 internal reference genes for data normalization	12 Reactions	XT-CSO-MSCC-12
Low RNA Input Kit	Kit for use with all Low RNA Input Primer Pools	48 Reactions	LOW-RNA-48
nCounter Master Kit (Max or FLEX Systems) Reagents and Cartridges	Reagents, cartridges, and consumables necessary for sample processing on nCounter MAX and FLEX Systems	12 Reactions	NAA-AKIT-012
nCounter SPRINT Cartridge 1 Cartridge, 12 Ianes	Sample Cartridge for nCounter SPRINT System	12 Reactions	SPRINT-CAR-1.0
nCounter SPRINT Reagent Pack	nCounter SPRINT Reagent Pack containing Reagents A, B, C, and Hybridization Buffer	192 Reactions	SPRINT-REAG-KIT

Gene Expression Panels arrive ready-to-use and generally ship within 24 hours following purchase.

Selected Panel References

- 1. Messmer, T. et al. Transcriptional Heterogeneity in Naive and Primed Human Pluripotent Stem Cells at Single-Cell Resolution. *Cell Rep* **26**, 815-824.e4 (2019).
- 2. Atlasi, Y. & Stunnenberg, H. G. The interplay of epigenetic marks during stem cell differentiation and development. *Nature Reviews Genetics* **18**, 643–658 (2017).
- Mallon, B. S. et al. StemCellDB: The Human Pluripotent Stem Cell Database at the National Institutes of Health. Stem Cell Res 10, 57–66 (2013).
- 4. Yamanaka, S. Pluripotent Stem Cell-Based Cell Therapy—Promise and Challenges. Cell Stem Cell 27, 523–531 (2020).
- 5. Cunningham, J. J., Ulbright, T. M., Pera, M. F. & Looijenga, L. H. J. Lessons from human teratomas to guide development of safe stem cell therapies. *Nature Biotechnology* **30**, 849–857 (2012).
- 6. Takahashi, K. & Yamanaka, S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. *Cell* **126**, 663–676 (2006).
- 7. Shi, Y., Inoue, H., Wu, J. C. & Yamanaka, S. Induced pluripotent stem cell technology: a decade of progress. *Nature Reviews Drug Discovery* **16**, 115–130 (2017).
- 8. Weinberger, L. Dynamic stem cell states: naive to primed pluripotency in rodents and humans. 15.
- 9. Koyanagi-Aoi, M. et al. Differentiation-defective phenotypes revealed by large-scale analyses of human pluripotent stem cells. *Proc Natl Acad Sci U S A* **110**, 20569–20574 (2013).
- Villa-Diaz, L. G., Ross, A. M., Lahann, J. & Krebsbach, P. H. Concise Review: The Evolution of human pluripotent stem cell culture: From feeder cells to synthetic coatings. STEM CELLS 31, 1–7 (2013).

To view the annotated gene lists for the Stem Cell Characterization Panel, visit <u>nanostring.com/stem-cell-characterization</u>

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