

STEMdiff™ Trilineage Differentiation Kit

Directed Differentiation for Clear, Reproducible Validation of Pluripotency

The new STEMdiff™ Trilineage Differentiation Kit provides a simple cell culture assay to functionally and reproducibly validate the ability of new or established human embryonic stem (ES) and induced pluripotent stem (iPS) cell lines to differentiate to the three germ layers: ectoderm, mesoderm and endoderm. This kit includes reagents and protocols to perform parallel in vitro directed differentiation experiments for each germ layer, clearly establishing trilineage differentiation potential within one week. Using this system, independent assays for each germ layer are carried out in separate wells, allowing for clarity in the experimental readout that is often lacking for in vitro spontaneous differentiation experiments; cells successfully directed to the endoderm lineage, for example, express endoderm markers and not ectoderm or mesoderm markers. Compared to traditional teratoma assays, this standardized, in vitro procedure enables faster, more cost-effective evaluation of pluripotency at significantly higher throughput.

Clear, quantitative assay results evaluated by immunocytochemistry, flow cytometry or transcriptome analysis make the STEMdiff™ Trilineage Differentiation Kit a valuable tool for establishing the pluripotency of a given cell line with the confidence required to carry it forward for further characterization or banking.

Why Use the STEMdiff™ Trilineage Differentiation Kit?

- ROBUST.** Reproducible directed differentiation to all three germ layers across multiple pluripotent cell lines.
- CLEAR.** Easy-to-interpret assay results.
- DEFINED.** Complete, defined cell culture media.
- EFFICIENT.** Standardized, one-week protocol.

PRODUCT	CAPACITY	CATALOG #
STEMdiff™ Trilineage Differentiation Kit	1 kit	05230

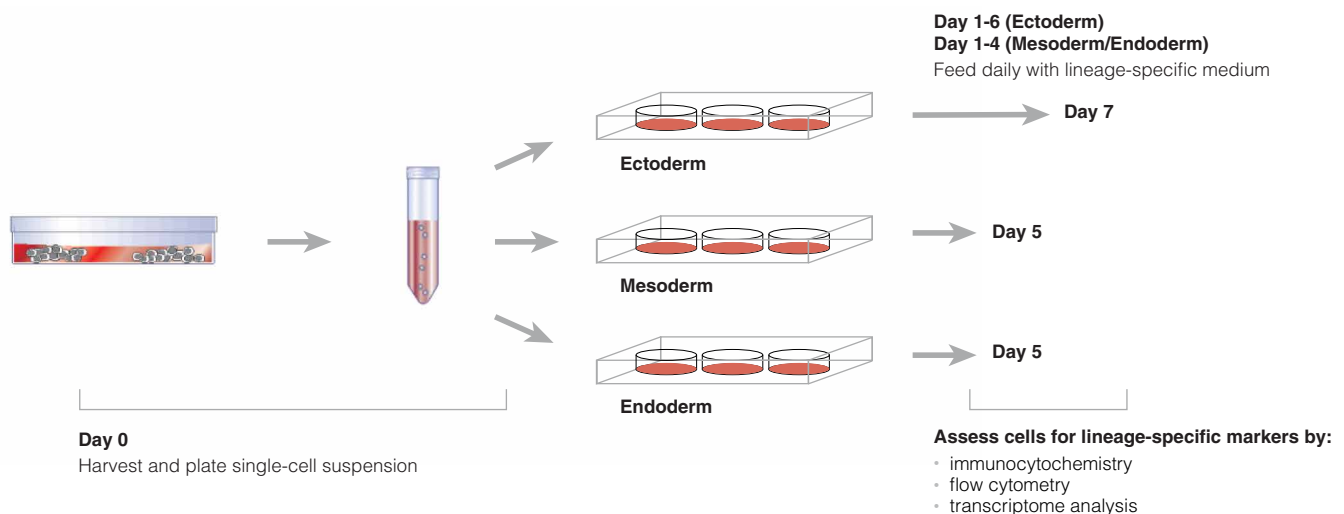


Figure 1. Schematic of STEMdiff™ Trilineage Differentiation Kit assay

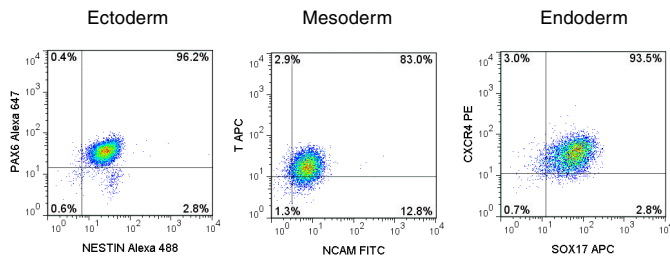


Figure 2. Flow cytometry analysis shows efficient differentiation to all three germ layers

H9 cells were maintained in mTeSR™1, differentiated in vitro using the STEMdiff™ Trilineage Differentiation Kit and subjected to flow cytometry analysis. This representative experiment shows efficient differentiation as evidenced by the double-positive expression pattern of lineage-specific markers for each germ layer.

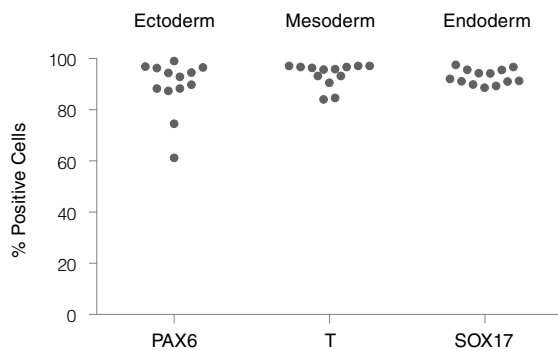


Figure 3. The STEMdiff™ Trilineage Differentiation Kit promotes efficient differentiation to all three germ layers

Human pluripotent stem cells (both iPS and ES cells represented) were maintained in mTeSR™1, differentiated using the STEMdiff™ Trilineage Differentiation Kit and subjected to flow cytometry analysis (N = 13 biological replicates including 5 distinct cell lines). The markers used for flow cytometry for each germ layer are listed below the X-axis.

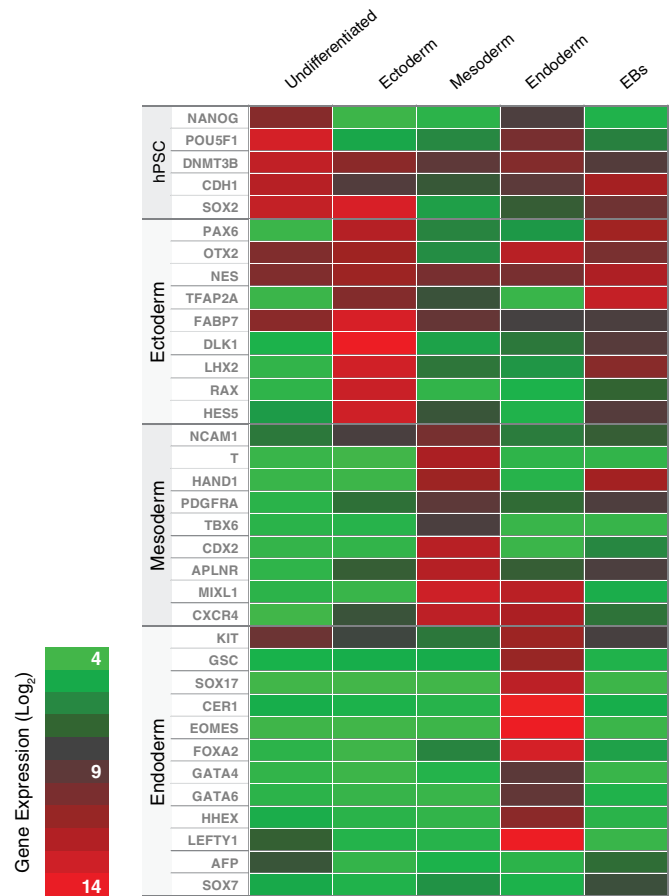


Figure 4. Molecular analysis of cultures differentiated with the STEMdiff™ Trilineage Differentiation Kit show strong separation of lineage-specific markers

H9 cells were maintained in mTeSR™1 and subsequently differentiated in vitro using either directed differentiation with the STEMdiff™ Trilineage Differentiation Kit or spontaneous differentiation in embryoid bodies (EBs) using a 10-day protocol in serum-containing medium. Undifferentiated cells, differentiated ectoderm, mesoderm and endoderm cells from the directed differentiation kit and EBs were then subjected to a microarray-based transcriptome analysis to evaluate expression levels of key germ layer markers. Cells differentiated using the STEMdiff™ Trilineage Differentiation Kit showed clear upregulation of appropriate germ layer-specific markers, whereas the same cells differentiated spontaneously in EBs did not show significant upregulation of mesoderm or endoderm makers.

For a complete list of related products, including specialized cell culture and storage media, matrices, antibodies, cytokines and small molecules, visit www.stemcell.com/hPSCworkflow or contact us at techsupport@stemcell.com.

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