

**Measuring energy metabolism in cultured cells, including human pluripotent stem cells and differentiated cells.**

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**Public Summary:**

Measurements of glycolysis and mitochondrial function are required to quantify energy metabolism in a wide variety of cellular contexts. In human pluripotent stem cells (hPSCs) and their differentiated progeny, this analysis can be challenging because of the unique cell properties, growth conditions and expense required to maintain these cell types. Here we provide protocols for analyzing energy metabolism in hPSCs and their early differentiated progenies that are generally applicable to mature cell types as well. Our approach has revealed distinct energy metabolism profiles used by hPSCs, differentiated cells, a variety of cancer cells and Rho-null cells. The protocols measure or estimate glycolysis on the basis of the extracellular acidification rate, and they measure or estimate oxidative phosphorylation on the basis of the oxygen consumption rate. Assays typically require 3 h after overnight sample preparation. Companion methods are also discussed and provided to aid researchers in developing more sophisticated experimental regimens for extended analyses of cellular bioenergetics.

**Scientific Abstract:**

Measurements of glycolysis and mitochondrial function are required to quantify energy metabolism in a wide variety of cellular contexts. In human pluripotent stem cells (hPSCs) and their differentiated progeny, this analysis can be challenging because of the unique cell properties, growth conditions and expense required to maintain these cell types. Here we provide protocols for analyzing energy metabolism in hPSCs and their early differentiated progenies that are generally applicable to mature cell types as well. Our approach has revealed distinct energy metabolism profiles used by hPSCs, differentiated cells, a variety of cancer cells and Rho-null cells. The protocols measure or estimate glycolysis on the basis of the extracellular acidification rate, and they measure or estimate oxidative phosphorylation on the basis of the oxygen consumption rate. Assays typically require 3 h after overnight sample preparation. Companion methods are also discussed and provided to aid researchers in developing more sophisticated experimental regimens for extended analyses of cellular bioenergetics.

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