iPSCs differentiation into each of the three germ layers (endoderm, mesoderm and ectoderm)

To determine if our cells are truly pluripotent, it is important to verify their ability to differentiate into each of the three germ layers (endoderm, mesoderm and ectoderm). In brief, approximately 9 x 10^3 cells were plated per well of 8-well chamber slide, and grown overnight at 37 °C and 5% CO_2, or until they reached 50% confluency. Then we used the Human Pluripotent Stem Cell Functional Identification Kit (SC027B, R&D Systems®) to differentiate into endoderm and ectoderm. In addition, to differentiate into mesoderm, we used the Differentiation Base Media Supplement (50X), that comes with this kit, with CHIR99021 supplement (STEMCELL™ Technologies), a WNT pathway activator that promotes direct mesoderm differentiation. We used the kit antibodies for immunocytochemical studies: Goat Anti-Human Otx2 Antigen Affinity-purified Polyclonal Antibody (ectoderm marker), Goat Anti-Human Brachyury Antigen Affinity-purified Polyclonal Antibody (mesoderm marker), and Goat Anti-Human SOX17 Antigen Affinity-purified Polyclonal Antibody (endoderm marker). Differentiated iPSCs were fixed in 4% paraformaldehyde (Sigma-Aldrich) for 10 minutes, incubated with phosphate-buffered saline solution with 0.1% Tween™ (PBST) and 1% BSA (Sigma-Aldrich) for 30 min and stained by standard immunofluorescence procedures. Cells were analyzed on DM400 M fluorescence microscope (Leica).