

# Human Three Germ Layer 3-Color Immunocytochemistry Kit

Catalog Number: SC022

Size: 25 Tests

## INTENDED USE

This product is designed for the immunocytochemical analysis of human pluripotent stem cells using the appropriate fluorochrome-conjugated antibodies to label cells that have differentiated into each of the three germ layers.

## PRODUCT DESCRIPTION

This kit contains six fluorochrome-conjugated antibodies that can be used for single-step immunocytochemical staining of human pluripotent stem cells differentiated into each of the three germ layers: ectoderm, mesoderm, and endoderm.

This package insert must be read in its entirety before using this product.  
For research use only. Not for use in diagnostic procedures.

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### MANUFACTURED AND DISTRIBUTED BY:

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MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C **in the dark**.  
Use within 6 months of receipt.

ECTODERM

PART	PART #	DESCRIPTION
Anti-human SOX1 NL493-Conjugated Goat IgG	967390	125 µL of a 10X solution in PBS containing < 0.1% sodium azide.
Anti-human Otx-2 NL557-Conjugated Goat IgG	967389	

MESODERM

PART	PART #	DESCRIPTION
Anti-human Brachyury NL557-Conjugated Goat IgG	967388	125 µL of a 10X solution in PBS containing < 0.1% sodium azide.
Anti-human HAND1 NL637-Conjugated Goat IgG	967392	

ENDODERM

PART	PART #	DESCRIPTION
Anti-human GATA-4 NL493-Conjugated Goat IgG	967391	125 µL of a 10X solution in PBS containing < 0.1% sodium azide.
Anti-human SOX17 NL637-Conjugated Goat IgG	967393	

SPECTRAL CHARACTERISTICS

The spectral characteristics of each of the fluorochromes used are described below.

Fluorochrome	Absorption Maximum (nm)	Emission Maximum (nm)
NL493	493	514
NL557	557	574
NL637	637	658

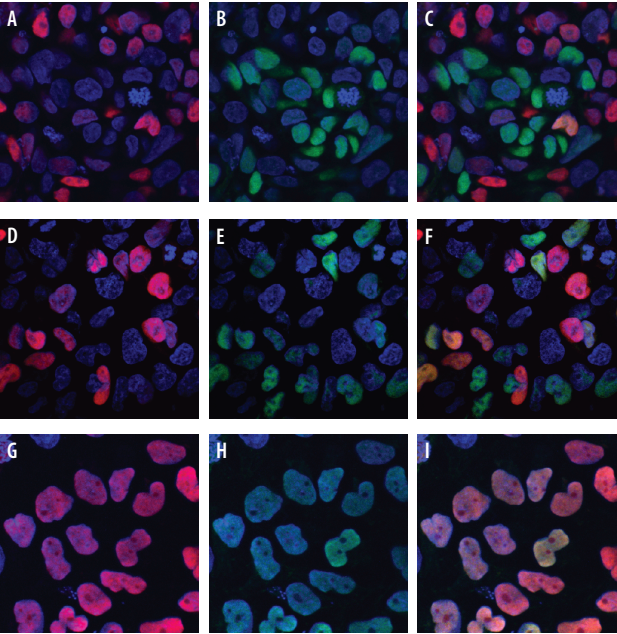
PRECAUTIONS

Sodium azide may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.  
  
Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

IMMUNOCYTOCHEMISTRY VALIDATION

These antibodies have been tested for immunocytochemical staining using human pluripotent stem cells differentiated into each of the three germ layers using the Human Pluripotent Stem Cell Functional Identification Kit (R&D Systems®, Catalog # SC027B). Cells were fixed in PBS containing 4% paraformaldehyde and blocked with PBS containing 10% normal donkey serum, 0.3% Triton™ X-100, and 1% BSA. After blocking, cells were incubated with two conjugated antibodies corresponding to the cell lineage of interest, each at a final concentration of 1X (1:10 dilution) in blocking buffer for 3 hours at room temperature **in the dark**. Cells were then washed with PBS containing BSA. When using a staining volume of 50 µL, this kit contains sufficient material for 25 tests of each antibody.

DATA EXAMPLES



**Figure 1:** iPS2 human induced pluripotent stem cells were differentiated into each of the three germ layers according to the Human Pluripotent Stem Cell Functional Identification Kit (R&D Systems®, Catalog # SC027B). Ectoderm differentiated cells were simultaneously stained with Northern Lights™ (NL) 557-conjugated Otx-2 (A and C; red) and NL493-conjugated SOX1 (B and C; green). Mesoderm differentiated cells were simultaneously stained with NL557-conjugated Brachyury (D and F; red) and NL637-conjugated HAND1 (E and F; green). Endoderm differentiated cells were simultaneously stained with NL637-conjugated SOX17 (G and I; red) and NL493-conjugated GATA-4 (H and I; green). All nuclei were stained with DAPI (blue).