#9094 Store at -20°C

StemLight™ Pluripotency Surface Marker Antibody Kit

☑ 1 Kit
(3 x 20 µl)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 06/16

For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	IF-IC Dilution	Isotype
*SSEA4 (MC813) Mouse mAb	4755	20 µl	1:500	Mouse IgG3
*TRA-1-60(S) (TRA-1-60(S)) Mouse mAb	4746	20 µl	1:1000	Mouse IgM
*TRA-1-81 (TRA-1-81) Mouse mAb	4745	20 µl	1:250	Mouse IgM

Description: The StemLight™ Surface Marker Kit contains a panel of antibodies for the detection of antigens that are specifically expressed on the surface of human pluripotent cells. The kit can be used to track the pluripotent potential of human embryonic stem (ES) or induced pluripotent (iPS) cells. The loss of these markers indicates a loss of pluripotency or differentiation of the culture. The kit components are pre-optimized for parallel use in immunofluorescent analysis, but components are also validated for use in other applications please refer to individual data sheet information for application specific recommendations.

Specificity/Sensitivity: TRA-1-60(S) (TRA-1-60(S)) Mouse mAb detects endogenous levels of the neuraminidase-sensitive TRA-1-60 antigen. TRA-1-81 (TRA-1-81) Mouse mAb detects endogenous levels of TRA-1-81 antigen. SSEA4 (MC813) Mouse mAb detects endogenous levels of SSEA4 antigen.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with human embryonic carcinoma 2102Ep cl.2A6 cells.

Background: Pluripotency is the ability of a cell to differentiate into cell types of the three germ layers, the endoderm, ectoderm and mesoderm. It is a property shared by embryonic stem cells, embryonic carcinoma and induced pluripotent cells.

SSEA4, TRA-1-81 and TRA-1-60 antibodies recognize antigens expressed on the cell surface of all pluripotent cells. SSEA4 recognizes a glycolipid carbohydrate epitope (1). TRA-1-60(S) and TRA-1-81 antibodies recognize different proteoglycan epitopes on variants of the same protein, podocalyxin (2). These epitopes are neuraminidase sensitive and resistant, respectively. Reactivity of SSEA4, TRA-1-81 and TRA-1-60 antibodies with their respective cell surface markers are lost upon differentiation of pluripotent cells, corresponding with a loss of pluripotent potential (3).

Background References:

(1) Henderson, J.K. et al. (2002) *Stem Cells* 20, 329-37.

(2) Draper, J.S. et al. (2002) *J Anat* 200, 249-58.

(3) Schopperle, W.M. and DeWolf, W.C. (2007) *Stem Cells* 25, 723-30.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:
The kit components are preoptimized for parallel use in immunofluorescent analysis at a standard dilution, but components are also validated for use in other applications - please refer to individual data sheet information for application specific recommendations.

*Note: Due to the extracellular location of the epitope, permeabilization (detergents or alcohols) should be omitted.

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

All components of this kit have been validated for reactivity with human protein, however some components also have additional species cross reactivities. Please see www.cellsignal.com for additional specificity/sensitivity information for individual kit components.

#9094

Immunofluorescence Protocol

***IMPORTANT:** Please refer to the **APPLICATIONS** section on the front page of the data sheet to determine **IF THIS PRODUCT** is validated and approved for the specific protocol you will be using.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. **10X Phosphate Buffered Saline (PBS):** To prepare 1 L add 80 g sodium chloride (NaCl), 2 g potassium chloride (KCl), 14.4 g sodium phosphate, dibasic (Na_2HPO_4) and 2.4 g potassium phosphate, monobasic (KH_2PO_4) to 1 L dH_2O. Adjust pH to 7.4.
2. Formaldehyde, 16%, methanol free, Polysciences, Inc. (cat# 18814), use fresh, store opened vials at 4°C in dark, dilute in PBS for use.
3. Xylene
4. Ethanol, anhydrous denatured, histological grade, 100% and 95%
5. Distilled water (dH_2O)
6. **Blocking Buffer:** To prepare 25 mL, add 2.5 mL 10X PBS, 1.25 mL normal serum from the same species as the secondary antibody (eg. normal goat serum, normal donkey serum) and 21.25 mL dH_20 and mix well. While stirring, add 75 µL Triton X-100 (100%).
7. **Antibody Dilution Buffer:** To prepare 40 mL, add 4 mL 10X PBS to 36 mL dH_2O, mix. Add 0.4 g BSA and mix well. While stirring, add 120 µL Triton X-100 (100%).
8. **10 mM Sodium Citrate Buffer:** To prepare 1 L, add 2.94 g sodium citrate trisodium salt dihydrate ($C_6H_5Na_3O7 \bullet 2H_2O$) to 1 L dH_2O. Adjust pH to 6.0.
9. **1X PBS, high salt (0.4M) (high salt PBS):** To prepare 1L, add 100 ml 10X PBS to 900 ml dH20. Add 23.38 g NaCl and mix.
10. Fluorochrome-conjugated secondary antibody

NOTE: When using any primary or fluorochrome-conjugated secondary antibody for the first time, titrate the antibody to determine which dilution allows for the strongest specific signal with the least background for your sample.

11. Prolong® Gold Antifade Reagent (Invitrogen, Eugene, OR, Cat# P36930)

B Specimen Preparation

I. Cultured Cell Lines (IF-IC)

IMPORTANT: Please check the **APPLICATIONS** section of the data sheet to verify that this product is validated and approved for **(IF-IC).**

NOTE: Cells should be grown, treated, fixed, and stained directly in multiwell plates, chamber slides, or on coverslips.

1. Rinse cells briefly in PBS.
2. Aspirate PBS, cover cells to a depth of 2-3 mm with 2-4% formaldehyde in PBS.

NOTE: Formaldehyde is toxic, use only in fume hood.

3. Allow cells to fix for 15 minutes at room temperature.
4. Aspirate fixative, rinse three times in PBS for 5 minutes each.
5. **Methanol Permeabilization Step (if required, please refer to front page):** After formaldehyde fixation, cover cells with ice-cold 100% methanol (use enough to cover cells completely to a depth of 3-5 mm, DO NOT LET CELLS DRY), incubate cells in methanol for 10 minutes at –20°C, rinse in PBS for 5 minutes.
6. Proceed with Immunostaining section C.

II. Paraffin Sections (IF-P)

IMPORTANT: Please check the **APPLICATIONS** section of the data sheet to verify that this product is validated and approved for **(IF-P).**

Deparaffinization/Rehydration:

1. Incubate sections in three washes of xylene for 5 minutes each.
2. Incubate sections in two washes of 100% ethanol for 10 minutes each.
3. Incubate sections in two washes of 95% ethanol for 10 minutes each.
4. Rinse sections twice in dH_2O for 5 minutes each.

Antigen Unmasking:

1. Place slides in room temperature 10 mM sodium citrate buffer pH 6.0.
2. Bring slides to boiling in sodium citrate buffer using water bath or microwave, then maintain at 95-99°C for 10 minutes.
3. Cool slides for 30 minutes on bench top.
4. Rinse sections in dH_2O three times for 5 minutes each.
5. Rinse sections in PBS for 5 minutes.
6. Proceed with Immunostaining section C.

III. Frozen/Cryostat Sections (IF-F)

IMPORTANT: Please check the **APPLICATIONS** section of the data sheet to verify that this product is validated and approved for **(IF-F).**

NOTE: Fresh frozen/unfixed sections should be fixed immediately in 2-4% formaldehyde as follows to preserve signaling epitopes.

1. Cover sections with 2-4% formaldehyde in PBS

NOTE: Formaldehyde is toxic, use only in fume hood.

2. Allow sections to fix for 15 minutes at room temperature.
3. Rinse slides three times in PBS for 5 minutes each.

C Immunostaining

NOTE: All subsequent incubations should be carried out at room temperature unless otherwise noted in a humid light-tight box or covered dish/plate to prevent drying and fluorochrome fading.

1. Block specimen in Blocking Buffer for 60 minutes.
2. While blocking, prepare primary antibody by diluting as indicated on datasheet in Antibody Dilution Buffer.
3. Aspirate blocking solution, apply diluted primary antibody.

NOTE: For double-labeling, prepare a cocktail of the primary antibodies at their appropriate dilution in Antibody Dilution Buffer.

4. Incubate overnight at 4°C.
5. Rinse three times in PBS for 5 minutes each.

OPTION: To decrease background stain, rinse in high salt PBS for two minutes between second and third PBS rinses. Be aware, this may reduce specific staining of some antibodies.

NOTE: If using primary antibodies directly conjugated with Alexa Fluor® fluorochromes, then skip to step C8.

6. Incubate specimen in fluorochrome-conjugated secondary antibody diluted in Antibody Dilution Buffer for 1-2 hours at room temperature in dark.

NOTE: For double-labeling, prepare a cocktail of fluorochrome-conjugated secondary antibodies at their appropriate dilutions in Antibody Dilution Buffer.

7. Rinse in PBS/high salt PBS as in step 5.
8. Coverslip slides with Prolong® Gold Antifade Reagent or apply just enough to cover cells in multiwell plate.
9. Seal slides by painting around edges of coverslips with nail polish.
10. For best results examine specimens immediately using appropriate excitation wavelength. For long term storage, store slides flat at 4°C protected from light.