Human Oct-3/4 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF1759

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Oct-3/4 in direct ELISAs and Western blots.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human Oct-3/4 Met1-Asn265 (Met262Leu) Accession # Q01860
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 μm filtered solution in PBS.

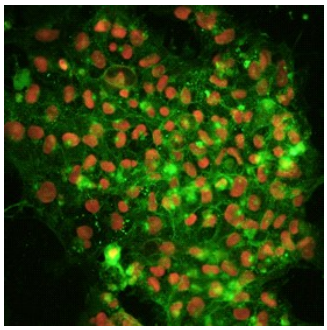
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.1 µg/mL	Recombinant Human Oct-3/4
Chromatin Immunoprecipitation (ChIP)	5 µg/10^6 cells	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Immunoprecipitation	3 µg/10^6 cells	NTera-2 human testicular embryonic carcinoma cell line, see our available Western blot detection antibodies

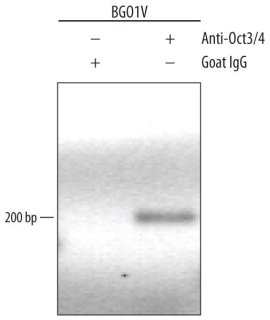
DATA

Immunocytochemistry



Alkaline Phosphatase and Oct-3/4 in BG01V Human Stem Cells. Alkaline phosphatase (ALPL) and Oct-3/4 were detected in human BG01V embryonic stem cells using 10 µg/mL Human/Mouse/Rat ALPL Monoclonal Antibody (Catalog # MAB1448) and 10 µg/mL Goat Anti-Human Oct-3/4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1759). Cells were incubated with primary antibodies for 3 hours at room temperature. Cells were stained for ALPL using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (pseudo-stained green; Catalog # NL007), and stained for Oct-3/4 using the NorthernLights 637-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL002). View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Chromatin Immunoprecipitation (ChIP)



Detection of Oct-3/4-regulated Genes by Chromatin Immunoprecipitation. BG01V human embryonic stem cells were fixed using formaldehyde, resuspended in lysis buffer, and sonicated to shear chromatin. Oct-3/4/DNA complexes were immunoprecipitated using 5 µg Goat Anti-Human Oct-3/4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1759) or control antibody (Catalog # AB-108-C) for 15 minutes in an ultrasonic bath, followed by Biotinylated Anti-Goat IgG Secondary Antibody (Catalog # BAF109). Immunocomplexes were captured using 50 µL of MagCellect Streptavidin Ferrofluid (Catalog # MAG999) and DNA was purified using chelating resin solution. The *nanog* promoter was detected by standard PCR.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Oct-3/4, a member of POU transcription factors, was identified as a DNA-binding protein that activates gene transcription via a cis-element containing an octamer motif (1). It is expressed in totipotent embryonic stem and germ cells (2, 3). A critical amount of Oct-3/4 expressed is required to sustain stem cell self-renewal and pluripotency (4). When embryonic stem cells are induced to differentiate, Oct-3/4 is downregulated and this downregulation of Oct-3/4 has proven to be essential for proper and divergent developmental program (5). The Oct-3/4 molecule is not only a master regulator of pluripotency that controls the lineage commitment but also is the most recognized marker used for the identification of totipotent embryonic stem cells.

References:

1. Scholer, H.R. *et al.* (1990) Nature **344**:435.
2. Scholer, H.R. *et al.* (1989) EMBO J. **8**:2543.
3. Rosner, M.H. *et al.* (1990) Nature **345**:686.
4. Niwa, H. *et al.* (2000) Nat. Genet. **24**:372.
5. Pesce, M. *et al.* (2001) Stem Cells **19**:271.