R&D® Ab
Research & Diagnostic Antibodies
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Rabbit Anti-TGF-Alpha Serum

IP-2516 Lot # 8357

The antiserum was raised in a rabbit which was immunized with a peptide analogue of the carboxyl terminal of the TGF-Alpha covalently attached onto a carrier protein. This antiserum is specific for the COOH terminal of the TGF-Alpha and is suitable for immunocytochemical detection.. The antiserum has been characterized ELISA, and cell staining techniques. Dilute the lyophilized antiserum with 0.1 ml of 10 mg/ml BSA is PBS for the equivalent of whole antiserum, or with additional buffer for more dilute antiserum.

Antiserum Specificity

Polypeptide	% Cross Reactivity
TFG-Alpha	100
Epidermal Growth Factor(Human)	0
	0
	0
	0
	0

Immunocytochemical Staining

This antiserum has been found to stain fixed cultured cells known to express hiNOS by indirect immunofluorescence at a dilution of 1:1000 to 1:5000.

Western Immunoblot

Western immunoblots using homogenate from cells expressing hiNOS resulted in a single band being detected at ~ 130 kDa at a dilution of 1:3000.

Western Blotting Protocol

1. After SDS-PAGE (on either 4-15% gradient gels or single percentage gels, such at 12% gels) and elecrophoretic transfer to PVDF membrane, block the membrane overnight with 4% normal goat serum in 1:5 diluted evaporated goat milk, using TBS/Tween-20 buffer as diluent.
2. Wash x 2 with TBS/Tween-20.
3. Apply the rabbit polyclonal antibody after dilution to at least 1:800(Note:higher dilutions may be needed). Use 1% normal goat serum in 1:5 diluted evaporated goat milk as buffer for the primary antibody. Dilute the condensed goat milk with TBS/Tween-20. Let the primary antibody bind for 2-4 hours.
4. Wash x 3 with TBS/Tween-20.
5. Apply affinity purified HRP-goat anti-rabbit IgG antiserum diluted 1:2500(dilution may vary depending upon supplier) in 1% normal goat serum in 1:5 diluted evaporated goat milk(useTBS/Tween-20 to dilute the goat milk). Incubate 1-2 hours. Note: greater sensitivity may be achieved using ABC techniques.
6. Wash x 3 and then soak the membrane overnight in a fairly large volume of TBS/Tween-20.
7. Develop color using the DAB reaction or the enhanced DAB reaction.