

**IgG Fraction of Rabbit Anti-Human Transforming Growth Factor  $\alpha$  Serum**

AS-2517

Lot # 2259

This antiserum was raised in a rabbit which was immunized with a synthetic analogue of the carboxyl terminal of human transforming growth factor  $\alpha$  (hTGF $\alpha$ ) covalently attached onto a carrier protein. This antiserum is specific for hTGF $\alpha$  and is suitable for immunocytochemical and western immunoblotting detection of this growth factor. The antiserum has been characterized by western blotting, ELISA, and cell staining techniques. Dilute the lyophilized antiserum with 0.1 ml of 10 mg/ml BSA in PBS for the equivalent of whole antiserum, or with additional buffer for more dilute antiserum.

**Antiserum Specificity****Polypeptide**

	<b>% Cross Reactivity</b>
Transforming Growth Factor $\alpha$ [33-50](human)	
TGF $\alpha$ (Human)	100
TGF $\alpha$ (rat)	100
EGF (human)	100
EGF (mouse)	0
Atrial Natriuretic Peptide (human)	0
ACTH (human, 1-39)	0
Calcitonin (human)	0
PTH (bovine, 1-84)	0
Somatostatin	0
Vasoactive Intestinal Peptide	0

**Immunocytochemical Staining**

This antiserum has been found to stain cells known to express human TGF $\alpha$  at a dilution of 1:800.

**Western Immunoblot**

Western immunoblots have been successful at a dilution of 1:1600.

**Western Blotting Protocol**

1. After SDS-PAGE on 15% gels and electrophoretic transfer to PVDF membrane, block the membrane overnight with 4% normal goat serum in TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. Apply the rabbit polyclonal antibody after dilution to at least 1:1600 (Note: higher dilutions may be needed). Use 2% normal goat serum in TBS/Tween-20 as buffer for the primary antibody. 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 2% normal goat serum in TBS/Tween-20. Incubate 1-2 hours. Note: greater sensitivity may be achieved using ABC techniques.
6. Wash x 4 for 5 minute per wash cycle with TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.