R&D ® Ab

Research & Diagnostic Antibodies

9030 W Sahara Ave. #403, Las Vegas, NV 89117 Phone: 702-638-7800

hiNOS Western Blot Standard from Induced DLD-1 Cells

ST-5202 Lot # 9091

This sample was prepared from DLD-1 cells (a human colorectal epithelial cell line) after induction with a mixture of cytokines. The induced cells were harvested, lysed by 2 freeze/thaw cycles, and the 16,000 x g supernatant boiled for 10 min after the additional of an equal volume of 2X SDS-PAGE sample buffer. Each vial contains 50 µl of prepared hiNOS standard. Use 5 - 10 µl per lane.

Western Blotting Protocol

1. After SDS-PAGE (on either 4 - 15% gradient gels or single percentage gels, such as 12% gels) and electrophoretic transfer to PVDF membrane, block the membrane overnight with 2% normal goat serum in TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. Apply the hiNOS specific polyclonal or monoclonal antibody after preparing the appropriate working dilution. 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-conjugated goat second antibody diluted to the appropriate working dilution (Note: 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 for 5 min x 4 in TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.

Western Blot Results

Western immunoblots result in a doublet band being detected at ~130 kDa with the lower molecular weight band being approximately 5,000 daltons less than the upper band. This is believed to result from cleavage at the amino terminal of the intact 1153 amino acid long protein during isolation.

Lane 1 = DLD-1 sample
Lane 2 = Mol. Wt. standards