

Antibody Protocols

1. Ki67 (for mouse not human)

Deparaffinize and hydrate formalin fixed paraffin embedded tissue sections in xylene or xylene substitute followed by graded alcohols (100%, 95% ETOH) to water.

Block endogenous peroxidase activity with 3% H₂O₂ in water for 10 minutes. Wash.

Antigen retrieve with 10mM Citrate Buffer pH 6.0 for 15 minutes in a microwave oven followed by a 20 minute cool down. Wash.

Block non-specific antibody binding by incubating slides with 10% rabbit serum in PBS for 30 minutes.

Drain slides and incubate with primary Ki67 antibody (Dako, #M7249) at a 1:200 dilution overnight at 4°C. Buffer wash.

Incubate slides with biotinylated rabbit-anti-rat IgG (Vector) at a 1:200 dilution for 30 minutes at room temperature. Buffer wash.

Incubate slides with ABC-HRP (Vector) for 30 minutes at room temperature. Buffer wash.

Incubate with DAB monitoring staining development.

Wash, counterstain, dehydrate, clear and coverslip slides for viewing.

2. 15LOX2

Deparaffinize and hydrate formalin fixed paraffin embedded tissue sections in xylene or xylene substitute followed by graded alcohols (100%, 95% ETOH) to water.

Block endogenous peroxidase activity with 3% H₂O₂ in water for 10 minutes. Wash.

Antigen retrieve with 10mM Citrate Buffer pH 6.0 for 10 minutes in a microwave oven followed by a 20 minute cool down. Wash.

Block non-specific antibody binding by incubating slides with 10% goat serum in PBS for 30 minutes.

Drain slides and incubate with primary 15LOX2 primary antibody (Oxford Biomedical) at a 1:1000 dilution for 30 minutes at room temperature. Buffer wash.

Incubate slides with Envision plus labeled polymer, anti-rabbit-HRP (Dako) for 30 minutes room temperature. Buffer wash.

Incubate with DAB monitoring staining development.

Wash, counterstain, dehydrate, clear and coverslip slides for viewing.

3. TUNEL staining follows the Intergen ApopTag® Peroxidase Kit #S7100.

