

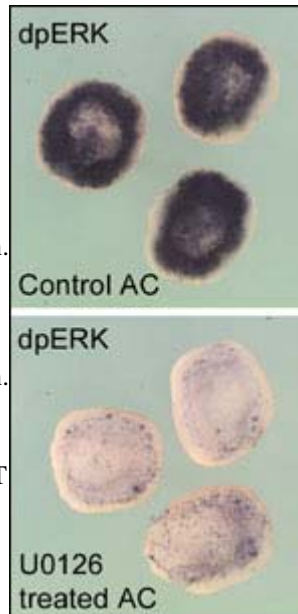
Wholemout immunostaining with Alkaline Phosphatase Antibodies

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Kuroda et al. *Genes Dev.* **19**, 1022-1027 (2005)

Immunostaining is done
using anti-dpERK mouse antibody (1:10,000) for the first antibody
and anti-mouse IgG-AP conjugated antibody (1:1,000) for the second antibody.
Conditions may change by different samples and antibodies.

- Fix embryos or explants in MEMFA for 2h at RT or for overnight in 4°C.
- Store in Methanol (MeOH) at -20° until use.
- Rehydrate into PBS.
- Treat with 0.1M K₂Cr₂O₇ in 5% acetic acid in PBS for 30 min.
- Wash with PBS for 30 min.
- Wash with 50% MeOH in PBS for 30 min.
- (Wash with 100% MeOH for 5 min) x 2.
- Treat with 5% H₂O₂ in MeOH for 1 hour.
- (Wash with 100% MeOH for 5 min) x 2.
- Wash with 50% MeOH in PBS for 30 min.
- Wash with PBS 3 time (total 2 h)
- Treat with BBT for 2h.
- Treat with First Antibody Solution in BBT for overnight in 4°C.
- Wash with BBT 4 times (total 4 h).
- Treat with 2nd Antibody Solution in BBT for overnight in 4°C.
- Wash with BBT for 1h.
- Wash with 0.1% Tween-20 in PBS for 4 hour.
- Wash with AP buffer 2-times (5 min each).
- Treat with BM purple at 4°C for 3 h-overnight (depending on sample).
- Stop with PBS.



Solutions:

BBT: 1% BSA and 0.1% Triton-X in PBS

AP buffer: 50 ml of Tris solution (1M Tris-HCl, 1M NaCl, pH 9.5), 50 ml of 0.5M MgCl₂ solution. Adjust volume to 500 ml with water..

BM purple (Roche)