BrdU incorporation in chick and mouse embryos - BrdU immunofluorescent labeling

For BrdU incorporation in mouse, inject pregnant female's intraperitonally (2 g/kg) 1 hour before sacrifice.

For BrdU incorporation in chick embryos, inject a single solution of 20 mg/ml of BrdU into the umbilical vein 30 minutes prior to collect the embryos (add fast green 0.1% to BrdU solution). Fix embryos and process them according to experimental procedure lab's before to embed and freeze in isopentane (at -55°C).

Incubate sections in PBS for 5 min and then incubate in HCl 2N for 30 min at room temperature. Wash extensively sections thrice in PBS (10 min) and apply blocking buffer on sections for 30 min (Blocking buffer : 1X PBS, 10% Horse serum heat-inactivated or Fetal bovine serum or BSA, 0.1% Triton). To process section according to experimental procedure lab's for immunofluorescent labeling:

- Saturate sections with blocking buffer for 30 min at room temperature.
- Incubate sections with primary antibodies (anti-BrdU at recommended dilution in blocking buffer) over night at 4°C.
- Wash sections thrice 5 min in 1X PBS 0.1% Triton at room temperature.
- Incubate sections with secondary antibodies (at recommended dilution in blocking buffer) for 30 min at room temperature.
- Wash sections thrice 5 min in 1X PBS + 0.1% Triton at room temperature.
- Final wash in 1X PBS + Hoechst or DAPI (1:25000).
- Mount slides with fluorescent mounting medium.
- Store slides at 4°C.