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EdU detection on tissue sections V.1

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol describes the procedure for EdU detection used by the Seifert Lab. It assumes a starting sample of a paraffin-embedded tissue section and refers to the Seifert Lab protocol for fluorescent immunohistochemistry [add link when published].

It is adapted from the procedure described in A. Salic, T.J. Mitchison, A chemical method for fast and sensitive detection of DNA synthesis *in vivo*, *Proc. Natl. Acad. Sci. U.S.A.* 105 (7) 2415-2420, https://doi.org/10.1073/pnas.0712168105 (2008).

Protocol materials

- Tris-buffered saline (TBS), 1x solution **Fisher Scientific** **Catalog #**BP24721
- Hoechst 33342 **Catalog #**H3570
- Prolong Gold **Thermo Fisher Scientific** **Catalog #**P36930

1 Deparaffinize and rehydrate slides per normal IHC protocol

2 If using subsequent antibodies for IHC, perform antigen retrieval

3 Rinse slides two times in
Tris-buffered saline (TBS), 1x solution **Fisher Scientific Catalog #**BP24721

4 *OPTIONAL*
Block for 00:05:00 to 00:10:00 in appropriate blocking solution

> Note
>
> This is not necessary for rodent ear or skin tissue

5 Leave slides in TBS until reaction cocktail is ready

6 Prepare EdU reaction cocktail:

	A	B	C
	Component	Volume (µL)	Notes
	2 M Tris, pH 8.5	5	100 mL = 24.2 g
	50 mM CuSO4	2	1 mL = 7.98 mg
	0.5 M ascorbic acid	20	1 mL = 88 mg *UNSTABLE AT RT, MAKE FRESH
	AlexaFluor Azide (0.25 mg/mL)	0.2	0.5 mg in 2 mL DMSO
	ddH2O	73	

	A	B	C
	Total Volume:	100	

Note

- **IMPORTANT**: use the reaction cocktail within 00:15:00 of preparing
- Use half the indicated AlexaFluor Azide to reduce background staining (0.1 µL per 100 µL
- 10 mM EdU = 2.55 mg/mL
- too much ascorbic acid messes up phalloidin staining
- 250 nM AlexaFluor azide (AF488) for staining of live cells is 0.45 µL/mL
- Use half the indicated AlexaFluor Azide to reduce background staining (0.1 µL per 100 µL)

7 Remove TBS and blot slides

8 Add reaction cocktail to each slide

Note

100 µL to 250 µL usually works well

9 Incubate for 00:30:00 at Room temperature protected from light

10 Wash slides two times for 00:05:00 each in TBS

11 **EITHER:**
Continue with normal fluorescent IHC protocol from blocking step

Note

IMPORTANT: keep slides protected from light for all steps to reduce bleaching of EdU signal

12 **OR**:

Counterstain nuclei by incubating with [M] 1 µg/mL Hoechst 33342 **Catalog #**H3570 for 00:05:00

12.1 Rinse 3 times with ddH_2O

12.2 Mount with Prolong Gold **Thermo Fisher Scientific Catalog #**P36930