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1) Here is the protocol I have been using for Phospho-ERK staining on cryosections.

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phospho-ERK Cell Signaling #9101 Rabbit (I have tried Cell Signal's mouse monoclonal without success).

Frozen sections (Tissue fixed in 4% PFA, stored in MeOH and rehydrated)

Cut 8-10 $\mu$ m sections

Dried for 1-2hrs before freezing sections in -80C

Thaw for at least 30min before using

Rehydrate through EtOH gradient (100, 90, 80, 70)

Wash in TBS x 3

Block in 3% BSA/TBS x 1hr RT

Wash in TBS x 1

O/N 1:100 anti-PhosphoERK in TBS 4C

Wash in TBS x 3

Apply 2 $^{\circ}$  anti-rabbit Alexa 488 (Molecular Probes)

1:100-200 in TBS x 30-60min RT or O/N 4C

Wash in TBS x 3

Counterstain in DAPI

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2) For vibrotome sections, the major variations are using TBS/0.1% Triton instead of TBS alone. And I block with 5% fetal calf serum in TBS/0.1% Triton