

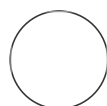


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# 🌐 Immunohistochemistry for p-eIF2a and eIF2a, v.230804

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We use this protocol and it's working

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## ABSTRACT

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## Reagents

1

phospho-eIF2a antibody: Abcam ab32157

eIF2a antibody: Cell signaling #5324

## Tissue Prep

2

Brains are stored in 4% PFA for 24hrs in 4C before storage in 1xPBS in 4C until sectioning  
Brain is sectioned in 1xPBS bath into 40um slices using Vibratome.  
Slices are stored in 0.03% Sodium Azide in 1xPBS in 24 well plates in 4C.

## IHC

3

In 24 well plate/ 2 slices per well with a minimum of 300uL of solution in well

4

Day 1:  
1. Alkaline Retrieval: 20 min in 1% NaOH (in 1x PBS) on shaker (RT) (make fresh each time, do not store in glass)

5

2. 3x10 min wash on shaker in PBS-T (PBS with 0.3% Triton X-100) (RT)

6

3. 2 hr blocking in 10% NDS/1% BSA in PBS-T (RT)

7

4. Primary antibody in dilution buffer (5% NDS +1%BSA) in PBS-T 48hrs in 4C on shaker

a. P-eIF2a Antibody (dilution 1:100 )

OR

b. eIF2a Antibody (dilution: 1:500 )

Day 2:

**8**

Day 2:

1. 3x10 min wash on shaker in PBS-T (RT)

**9**

2. Secondaries for 2hrs in dilution buffer (5% NDS +1%BSA) in PBS-T (RT)

● DK~Rb Alexa fluor 488+ (1:500)

**10**

3. 3x10 min wash on shaker in PBS-T (RT)

○ (for Hoescht: 1:750 in 1st 10 min wash)

**11**

4. Mount on slides by floating section in PBS and let sit until dry

**12**

5. Coverslip with Vectashield hardset mounting medium

**13**

6. Seal slide with nail polish next day

**14**

7. Store in 4C