

DeepLabel™ Antibody Staining Kit

C33001

Storage

4°C

- ✓ DeepLabel™ Solution A
- ✓ DeepLabel™ Solution B
- ✓ X-CLARITY™ Mounting Solution

Room temperature

- ✓ DeepLabel™ Washing Buffer

Product Description

C33002	DeepLabel™ Solution A
C33003	DeepLabel™ Solution B
C33004	DeepLabel™ Washing Buffer
C13101	X-CLARITY™ Mounting Solution

DeepLabel™ Antibody Staining Kit is a set of reagents optimized for use with clarified tissues for effective antibody penetration and site-specific binding.

DeepLabel™ Solution A is a permeabilization reagent that enhances antibody permeation. **DeepLabel™ Solution B** is an antibody dilution buffer that facilitates the antigen-antibody binding reaction.

Unbound antibodies are efficiently removed by the **DeepLabel™ Washing Buffer**. **X-CLARITY™ Mounting Solution** is a refractive index matching solution (RIMS) that minimizes photobleaching and preserves fluorescence signals. The refractive index (RI) of the solution is 1.460 at 25°C and is stable over a wide temperature range.

Directions for Use

WITH CLARITY/PACT/CUBIC SAMPLES

**All steps should be performed at 37°C with gentle shaking.*

1. Wash cleared samples with 1X PBS for 24 hours. Replace PBS at least 3 times. This step is crucial to remove residual clearing reagents that can inhibit antigen-antibody binding.
2. Incubate in DeepLabel™ Solution A for 1-4 days. Times will need to be optimized according to tissue size and type.

! For CLARITY or PACT samples: Adjust incubation time in Solution A according to the acrylamide concentration used for tissue-hydrogel hybridization. For a whole mouse brain, 1% acrylamide will require 1 day of permeabilization and 4% acrylamide will require 4 days.

3. Incubate with primary antibody diluted in DeepLabel™ Solution B for 1-3 days. Begin with a 1:50-1:100 dilution if optimal antibody concentration has not determined. Optimal incubation time should be determined empirically.

! For 1-2 mm mouse brain slices, incubate for 1-2 days. For a whole mouse brain, incubate for 3 days.

! Exceeding 5 days may damage tissues.

4. Wash 3 times with DeepLabel™ Washing Buffer.
5. Incubate with secondary antibody diluted in DeepLabel™ Solution B for 1-3 days. Optimal incubation time should be determined empirically.
6. Wash 3 times with DeepLabel™ Washing Buffer.
7. Incubate in enough X-CLARITY™ Mounting Solution to fully submerge samples for 2-3 hours in the dark.
8. Incubate in fresh X-CLARITY™ Mounting Solution for an additional 1-2 hours in the dark.

! For optimal fluorescence imaging, avoid prolonged incubation in X-CLARITY™ Mounting Solution. Image as soon as the sample is ready.



HEADQUARTERS

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WITH iDISCO™ SAMPLES

**All steps should be performed at 37°C with gentle shaking.*

1. Wash fixed samples with 1X PBS for 24 hours. Replace PBS at least 3 times.
2. Incubate in DeepLabel™ Solution A for 3-7 days. Optimal incubation time should be determined empirically.
3. Incubate with primary antibody diluted in DeepLabel™ Solution B for 1-3 days. Begin with a 1:50-1:100 dilution if optimal antibody concentration has not determined. Optimal incubation time should be determined empirically.
4. Wash 3 times with DeepLabel™ Washing Buffer.
5. Incubate with secondary antibody diluted in DeepLabel™ Solution B for 1-3 days. Optimal incubation time should be determined empirically.
6. Wash 3 times with DeepLabel™ Washing Buffer.
7. Continue clearing with the iDISCO method.

WITH 10-100 μm TISSUE SECTIONS OR CELL SAMPLES

1. Fix and prepare samples according to standard IHC protocols.
2. Rinse slides 3 times with 1X PBS at room temperature.
3. Apply 300-500 μL DeepLabel™ Solution A and incubate at room temperature for 60 minutes.
4. Replace with primary antibody diluted in DeepLabel™ Solution B and incubate at 4°C overnight.

! For a stronger reaction, try step 4 at 37°C for 1-2 hours.

5. Rinse slides 3 times with DeepLabel™ Washing Buffer at room temperature.
6. Apply secondary antibody diluted in DeepLabel™ Solution B and incubate at room temperature for 60 minutes.
7. Rinse slides 3 times with DeepLabel™ Washing Buffer.
8. Mount with a coverslip and image.



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Disclaimer

This product is for research use only.

Please consult the material safety data sheet for information regarding hazards and safe handling practices.

References

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