

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.



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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.

Disclaimer

This product is for research use only.

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

References

1. Lee, E. et al. ACT-PRESTO: Rapid and consistent tissue clearing and labeling method for 3 dimensional (3D) imaging. *Sci Rep* 6, 18631 (2016).
2. Yang, B. et al. Single-cell phenotyping within transparent intact tissue through whole-body clearing. *Cell* 158, 945–958 (2014).
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4. Tainaka, K. et al. Whole-body imaging with single-cell resolution by tissue decolorization. *Cell* 159, 911–924 (2014).
5. Renier, N. et al. iDISCO: A simple, rapid method to immunolabel large tissue samples for volume imaging. *Cell* 159, 896–910 (2014).



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