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Product information: HAK-actin™ (SC071)

Actin probe for Ultrastructure Expansion Microscopy (U-ExM) based techniques

Introduction

HAK-actin™ is a pan-species Expansion Microscopy (ExM) compatible probe for actin visualization using U-ExM based protocols. HAK-actin™ binds specifically to F-actin. Its design allows HAK-actin™ to be anchored into the gel and expand faithfully to the original actin structure after the swelling step. HAK-actin™ contains an HA tag epitope, it can therefore be detected post expansion using a pair of anti-HA and species specific secondary antibodies. It combines simplicity through a single protocol and versatility as the fluorophore label on the secondary antibody can be chosen freely. Finally, the primary + secondary antibody boosts fluorescent signal amplification, circumventing volumetric dilution of the fluorescent signal due to expansion.

Contains 1 tube of HAK-actin™ (5 nmol) for 100 gels*.

How does HAK-actin™ work?

The HAK-actin™ probe contains 3 key elements:

1. A pan-species, highly specific, high affinity F-actin ligand for a robust binding to polymerized actin (F-actin) only.

2. An anchoring moiety that specifically reacts with acrylamide and covalently links HAK-actin™ to the expansion gel.

3. The HA-tag epitope sequence for a strong and specific recognition by anti-HA antibodies, even after expansion.

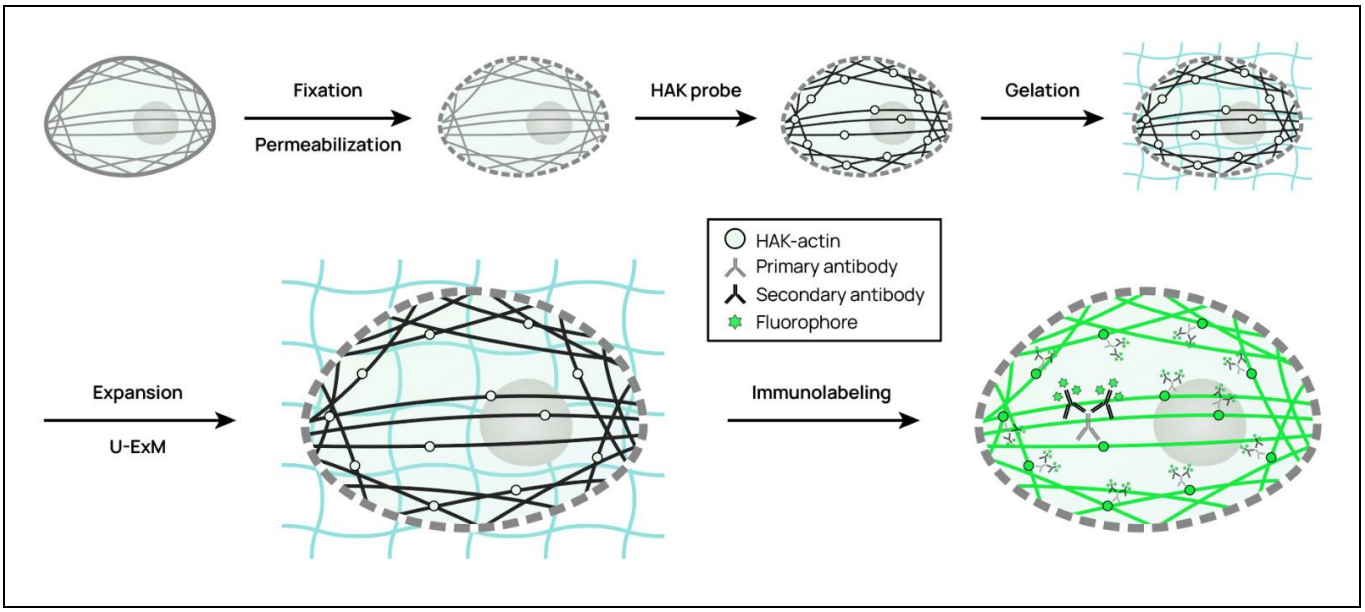

Figure 1. After fixation (chemical or cryo) and permeabilization, the sample to be expanded is treated with HAK-actin™. The probe's actin ligand uniformly labels F-actin within the cells. The sample is then subjected to U-ExM (or iU-ExM) protocol. During the protocol, HAK-actin™ is covalently bound within the expansion gel and expands faithfully with the sample. The final immunolabeling step using a primary anti-HA and a fluorescently labeled secondary antibody boosts the fluorescent signal and reveals the expanded actin structure with high contrast.

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Storage & Handling

Store HAK-actin™ at -20°C or below upon receipt. The compound is stable for >2 weeks at room temperature and for at least 12 months at -20°C. Reconstitute the probe using anhydrous DMSO. We recommend using newly or freshly opened and anhydrous DMSO to prepare the stock solution. Keep the stock solution of HAK-actin™ below -20°C after use. Vials should be allowed to warm to room temperature before opening. When reconstituted and stored properly, the stock solution is stable for 6 months. Note: DMSO solutions should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. Dispose of these reagents in compliance with all pertaining local regulations.

Labelling Protocol

Note: HAK-actin™ has been specifically designed for U-ExM, iU-ExM and Cryo-ExM protocols, other ExM methods might not work. The detailed protocols of these compatible expansion microscopy methods can be found in the following publications:
U-ExM: https://doi.org/10.1016/bs.mcb.2020.05.006

iU-ExM: https://doi.org/10.1038/s41467-023-43582-8

Cryo-ExM: https://doi.org/10.1038/s41592-021-01356-4

1. Prepare HAK-actin™ DMSO stock solutions.

Add 50 µL of anhydrous DMSO in the HAK-actin™ vial to prepare a **1000x stock solution** (DMSO stock solution HAK-actin™ concentration = 100 uM). After use, the DMSO stock solution should be stored at -20°C or below. It is not recommended to divide the DMSO stock solution into small aliquots, HAK-actin™ is not altered by multiple freeze-thaw cycles. When stored properly, the stock solution is stable for 6 months or more.

2. Cells fixation and HAK-actin™ treatment

1. Grow cells on sterile 12 mm coverslips until desired confluency is reached.
2. Gently wash coverslips with phosphate-buffered saline (PBS) to remove culture media.
3. Fix cells with 4% paraformaldehyde (PFA) in PBS for 5 minutes at room temperature (RT).
4. Incubate coverslips in PBS with 0.2% Tween-20 for 10 minutes at RT.
5. Incubate permeabilized cells with 1x HAK-actin™ (= 1ul of 1000x DMSO stock solution + 999 ul PBS/0.2% Tween) for 1 hour at RT.
6. Optional: incubate coverslips in a fixative solution of 4% PFA + 0.0125% glutaraldehyde (GA) for 15 minutes at RT.
7. Perform a quick wash with PBS.
8. Continue with Ultrastructure Expansion Microscopy (U-ExM) processing. For detailed protocols see the following publications.

   U-ExM: https://doi.org/10.1016/bs.mcb.2020.05.006

   iU-ExM: https://doi.org/10.1038/s41467-023-43582-8

   Cryo-ExM: https://doi.org/10.1038/s41592-021-01356-4

Note that for cryo-ExM, the probe can be added during the freeze-substitution or post-freeze substitution. 1x HAK-Actin™ (100nM final) is either added in acetone in the freeze substitution process or after freeze substitution and permeabilization, in PBS/Tween 0.2%, for 1h. Then, samples can be processed for U-ExM.

3. Immunolabeling

After expansion, the gels can be labelled using a primary anti-HA antibody and a fluorescently labelled secondary antibody following standard U-ExM immunolabeling protocol found here: https://doi.org/10.1016/bs.mcb.2020.05.006.

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The following anti-HA antibodies of different species origin were validated for U-ExM expanded samples:

Antibody	Supplier	Cat. number
Rabbit anti-HA (C29F4)	*Cell Signaling*	*3724*
Rat anti-HA (3F10)	*Roche*	*11867423001*
Mouse anti-HA Monoclonal (12CA5)	*Invitrogen*	*#MA1-12429*
Guinea Pig anti-HA	*ABCD antibodies*	*AF291*

4. References

Manuscript describing the HAK-actin™ on BioRxiv: https://doi.org/10.1101/2025.08.26.672318

U-ExM protocol: https://doi.org/10.1016/bs.mcb.2020.05.006

iU-ExM protocol: https://doi.org/10.1038/s41467-023-43582-8

Cryo-ExM protocol: https://doi.org/10.1038/s41592-021-01356-4

* Based on the following conditions: 0.5 ml staining solution / staining experiment with 1x probe concentration. The number of staining experiments can be further increased by reducing the staining volume.