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Product information: HAK-actin™ (SC019)

Actin probe for Ultrastructure Expansion Microscopy (U-ExM) and Cryofixation-Expansion Microscopy (Cryo-ExM)

Introduction

HAK-actin™ is a pan-species Expansion Microscopy compatible probe for actin visualization using either U-ExM or Cryo-ExM 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*.

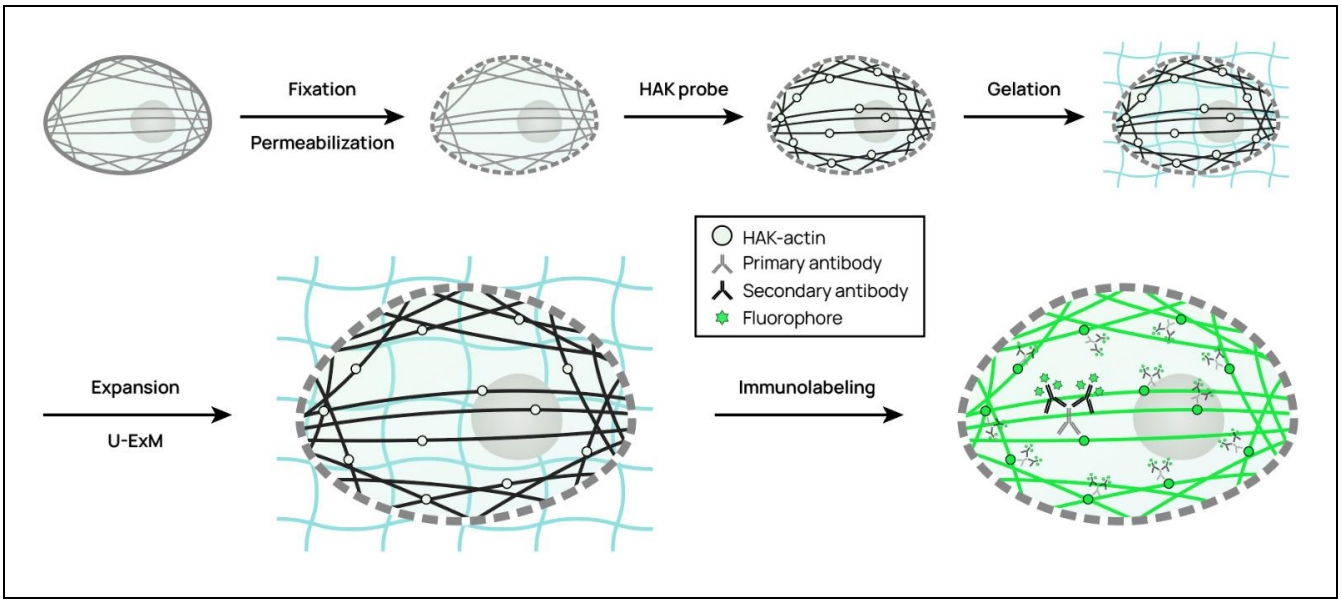
How does HAK-actin™ work?

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.



After fixation 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, Cryo-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.

Storage & Handling

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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. 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. Prepare the staining solution. Dilute the desired probe DMSO stock solution 1:1000 in your usual cell culture medium (e.g. DMEM + 10% fetal bovine serum) and vortex briefly. Proceed quickly to step 3. If the dilution is not performed in a single step, please use DMSO to prepare the intermediate dilution as using aqueous buffers to prepare the intermediate dilution may lead to the formation of aggregates. Since staining efficiency can depend on the cell line, it is recommended to stain cells with 1:1000 dilution at the first attempt and then optimize the dilution factor in further experiments until an optimal staining is achieved. The usual dilution facto for live cell labelling is 500-5000x. Use only freshly made staining solution and do not use it multiple times.

3. Cell preparation and staining. Grow cells on coverslips, glass bottom dish or glass bottom multi-well plates as usual. When cells have reached the desired density, replace the culture medium by the **staining solution** freshly prepared under step 2 ensuring that all the cells are covered with the solution. Place the cells in the incubator at 37°C in a humidified atmosphere containing 5% CO_2 for 1-2h (minimum 1h incubation).

Note: Before imaging, cells stained with SiR-actin or SPY650-FastAct can be fixed (PFA or GA fixation) and PFA or GA fixed cells can be stained with SiR-actin or SPY650-FastAct**. SPY650-FastAct_X can not be used with fixed cells.**

4. Cell imaging. Imaging of labeled cells is best performed using Cy5 settings. After labelling, the live cells can be immediately imaged without the need for washing steps. Optionally, a simple washing step consisting of replacing once the labelling solution by fresh culture medium which does not contain the probe may improve the signal to noise ratio in the case of SiR-actin and SPY650-FastAct. **It is not recommended to perform a washing step with SPY650-FastAct_X.**

* 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 volume or probe concentration.

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