Histone-Label for Immunostaining of Chromatin

For immunofluorescence of chromatin in fixed and permeabilized cells.
Only for research applications, not for diagnostic or therapeutic use.

1. Introduction

Histone-Label is a fluorescent chromatin probe for direct immunostaining of nuclei and chromosomes in fixed and permeabilized cells.

Histone-Label contains a small antigen-binding region of a camelid single-domain antibody (VHH), specifically binding to histone H2A-H2B heterodimers. The recombinant purified VHH is chemically conjugated to the fluorescent dye ATTO488 (from ATTO-TEC). Immunofluorescence staining with alpaca Nano-Boosters does not require any secondary antibody. Due to their small size, alpaca Nano-Boosters show better tissue penetration and improved staining precision, which allows obtaining higher resolution images.

2. Content

Reagent	Quantity	Code
Histone-Label_ATTO488	100 µl	tba488-100
Histone-Label_ATTO488	10 µl	tba488-10

Concentration: 0.5 – 1 mg/ml. Storage buffer: 1x PBS, 0.09% sodium azide.

3. Optical properties

ATTO 488: Excitation range 480 - 510 nm (λ_{abs}= 501 nm)
Emission range 520 - 560 nm (λ_{fl}= 523 nm)

For further information, please refer to *www.atto-tec.com.*

4. Stability and storage

Shipped at ambient temperature. Upon receipt store at +4°C.
Stable for 6 month. Do not freeze. Protect from light.

5. Protocol

1. **Fixation**: Fix cells in 3.7% formaldehyde in PBS for 10 min at room temperature.

   Note: Always prepare a fresh formaldehyde dilution.
2. Wash samples three times with PBS (phosphate buffered saline). Do not store fixed cells.
3. **Permeabilization:** Add PBS containing 0.5% Triton X-100 to samples and incubate for 5 min at room temperature.
4. Wash samples twice with PBS.
5. **Blocking**: Add 4% BSA in PBS to samples and incubate for 10 min at room temperature.
6. **Histone-Label incubation**: Dilute Histone-Label 1:400 in blocking buffer. Incubate cells with the diluted Booster in a humidified chamber for 1 h at room temperature.

   Note: Optimal working concentration is application-dependent and should be determined by testing the range of dilutions from 1/200 to 1/1000.

   Note: For multiplexing protocols, you can combine Histone-Label with another primary or secondary antibody.
7. Wash samples three times for 5-10 min in PBS.
8. **Recommended:** For better signal preservation, post-fix the staining with 3.7% formaldehyde in PBS for 10 min at room temperature. Wash samples three times with PBS.
9. **Imaging:** Proceed with imaging of the samples in PBS or imaging buffer within 3 days after completing the staining.
10. **Optional:** If mounting of samples is required, wash samples with PBS, rinse in H2O and mount in VECTASHIELD®. Caution: Mowiol® or ProLong® Diamond Antifade Mountant are not recommended for Histone-Label immunostained samples.

Suggested buffer composition

Buffer	Composition
Fixation buffer	3.7% formaldehyd in PBS
Permeabilization buffer	PBS; 0.5% Triton X-100
Wash buffer	PBS
Blocking buffer	4% BSA (w/v); PBS

Support/ Troubleshooting

Please refer to our FAQ section at *www.chromotek.com* or contact *support@chromotek.com.*

Related products

VHH Toolbox	Code
Histone-Chromobody	tcg
Actin-Chromobody	acg, acr
Lamin-Chromobody	lcg
GFP-Booster	gta488-10, gta488-100
GFP-Trap®_A	gta-20; gta-100; gta-200; gta-400