Vimentin-Label for Immunostaining of Vimentin Intermediate Filaments

For immunofluorescence of vimentin intermediate filaments in fixed cells.
Only for research applications, not for diagnostic or therapeutic use.

1. Introduction

The vimentin filament network plays a key role in cell architecture and signaling in mesenchymal cells. Vimentin is also an important marker of the epithelial–mesenchymal transition, a highly dynamic cellular process involved in the initiation of metastasis and cancer progression.

Vimentin-Label is a small vimentin-binding protein derived from a camelid VHH / so-called nanobody and conjugated to the fluorescent dye ATTO 488 (from ATTO-TEC). Immunofluorescence staining with alpaca Nano-Boosters & Nano-Labels is compatible with different fixation protocols and does not require any secondary antibody. Due to their small size, alpaca Nano-Boosters & Nano-Labels show better tissue penetration and improve staining precision, which allows obtaining higher resolution images.

2. Content

Reagent	Quantity	Code
Vimentin-Label_ATTO488	100 µl	vba488-100
Vimentin-Label_ATTO488	10 µl	vba488-10

Concentration: 0.3 - 0.5 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:** Wash cells seeded on coverslips twice with PBS (Phosphate Buffered Saline). Fix in 3.7% formaldehyde in PBS for 10 min at room temperature.

   Note: Always prepare a fresh formaldehyde dilution.
   Note: Alternatively, it is possible to use methanol or acetone:methanol for fixation: apply ice-cold 100% methanol or 1:1 acetone:methanol onto cells for 5 min.

2. Wash samples three times with PBS. Do not store fixed cells.
3. **Permeabilization and blocking:** Add PBS containing 0.2% Triton X-100 and 3% BSA to samples and incubate for 1 h at room temperature.
4. Wash samples three times with PBS.
5. **Vimentin-Label incubation:** Dilute Vimentin-Label 1:200 in PBS supplemented with 3% BSA. Apply onto samples and incubate overnight at 4°C.

   Note: For multiplexing protocols, you can combine Vimentin-Label with other primary or secondary antibodies.

6. Wash samples three times for 5-10 min in PBS.
7. If required, counterstain with DNA fluorescent dyes, e.g. DAPI in PBS. Wash samples with PBS. Proceed with imaging of the samples in PBS or imaging buffer.
8. **Post-fixation and mounting:** Alternatively, if mounting of samples is required, post-fix samples with 3.7% formaldehyde in PBS for 10 min at room temperature. Wash samples three times with PBS and mount in a medium of choice (Mowiol®, VECTASHIELD®, ProLong® Diamond Antifade Mountant, or other).

Suggested buffer composition

Buffer	Composition
Fixation buffer	3.7% formaldehyd in PBS
Permeabilization and blocking buffer	0.2% Triton X-100 (w/w); 3% BSA (w/v); PBS
Wash buffer	PBS
Immunostaining buffer	3% 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
Vimentin-Chromobody	vcg
Actin-Chromobody	acg, acr
Lamin-Chromobody	lcg
GFP-Booster	gta488-10, gta488-100
GFP-Trap®_A	gta-20; gta-100; gta-200; gta-400