

MULTIPLEXING

MASSIVE-sdAB-FAST PLEX kit

Pre-mixing protocol

General information about MASSIVE-sdAB-FAST 1- to 6-PLEX kit found in datasheet.

GENERAL PRE-MIXING PROTOCOL: PRE-MIXING OF SECONDARY SINGLE-DOMAIN ANTIBODIES (SDABS) WITH PRIMARY ANTIBODIES FOR SPECIES INDEPENDENT IMMUNOLABELING

General Protocol

- In separate tubes, add a 2.5- to 3-fold excess of secondary sdAB to each primary antibody. This will result in a 1.25- to 1.5-fold excess of secondary sdAB over available binding sites considering that there are two identical binding sites for the sdAB on each primary antibody. Perform pre-incubation in low volume (15 - 30 μ L) of Antibody Incubation Buffer per tube for at least 30 min at room temperature.
E.g., add 4 μ L secondary sdAB (= 20 pmol; stock concentration 5 μ M) per microgram of primary antibody (1 μ g of primary antibody = 6.7 pmol; M_r (IgG) = 150 kDa).
- Optional: add unlabeled sdABs with 2-fold molar excess to primary antibodies, and incubate for 2 min at room temperature.
- Pool all pre-mix solutions and if needed add Antibody Incubation Buffer to the desired final volume for the immunostaining the sample.
- Incubate solution on sample for at least 60 mins at RT. We found overnight incubation at 4 $^{\circ}$ C to be a good starting point (incubation time to be optimized for cell types and targets).
- Wash 3x with Washing Buffer.
- Postfixation with 4 % PFA for 5-15 min RT.
- Optional: Quench sample at RT:
 - Incubate sample with 0.2 M NH_4Cl or for 5 min OR.
 - Incubate sample with 1 mg/mL NaBH_4 for 5 min (Dissolve powder in PBS directly before use).
Prepare solution in 15 mL Falcon - don't close the lid after dissolving!
- Wash 3x with Washing Buffer.
- Add fiducials for channel alignment and drift correction. We recommend 90 nm gold nanoparticles (Cytodiagnostics, Cat. No G-90-20).
- Add imaging solution and perform DNA-PAINT imaging.

Notes

- Primary antibodies should be screened for specificity and functionality before performing pre-mixing.
- Certain cellular targets prefer different fixations. We recommend optimizing fixation/sample preparation conditions for multi-target imaging before trying DNA-PAINT.
- sdABs developed and manufactured by NanoTag Biotechnologies are sold under license. Unmodified and fluorophore- coupled sdABs (FluoTag®) remain available for direct purchase from NanoTag Biotechnologies.

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