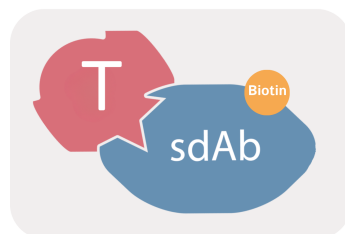


## Reconstitution and Storage

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## Biotinylated sdAbs

Biotinylated sdAbs are alpaca-derived single-domain antibodies (sdAbs) conjugated to a single biotin moiety at the C-terminus. These target-specific sdAbs can be applied across various methods utilizing streptavidin or avidin-based detection (e.g., immunoprecipitation, immunofluorescence, ELISA).



**Sketch of a biotinylated sdAb in complex with its target protein.**

**T:** target protein  
**sdAb:** single-domain antibody

Biotinylated sdAbs are **lyophilized from PBS pH 7.4** and shipped as a powder at ambient temperature. The lyophilized reagent can be stored at 2-8°C for up to 12 months.

After reconstitution in 250 µL, the final concentration of biotinylated sdAb is 1 mg/mL. For optimal performance, store the reconstituted reagent in aliquots at -80°C.

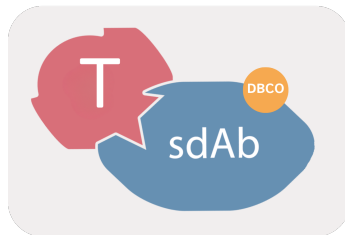
### Protocol: Reconstitution of Biotinylated sdAbs

1. Prepare sterile 50% glycerol (v/v) in deionized water.  
If applicable, we recommend including 0.1% sodium azide as a preservative. Sodium azide should be avoided when staining live cells or conducting *in vivo* studies.
  2. Open the vial containing the lyophilized biotinylated sdAb.
  3. Add 250 µL of sterile 50% glycerol (v/v) in deionized water.
  4. Mix gently and allow to sit at room temperature for ~5 min.
  5. Optional: Briefly spin down the vial for 2 min at 100 *xg* using a 50 mL conical tube with tissue paper at the bottom.
  6. Distribute into aliquots. Use small tubes and avoid aliquots below 20 µL.
  7. Storage:
    - Short-term: Working aliquot can be stored at -20°C for up to 4 weeks.
    - Long-term: Ideally store at -80°C (up to 6 month).
- Note:
- Avoid repeated freeze-thaw cycles.

**Only for research applications, not for diagnostic or therapeutic use**

## DBCO-conjugated sdAbs

DBCO-conjugated sdAbs are alpaca-derived single-domain antibodies (sdAbs) conjugated to a single dibenzocyclooctyne (DBCO) moiety at the C-terminus. These target-specific sdAbs can be applied across various methods employing click chemistry.



### Sketch of a DBCO-conjugated sdAb in complex with its target protein.

**T:** target protein

**sdAb:** single-domain antibody

**DBCO:** dibenzocyclooctyne

DBCO-conjugated sdAbs are **lyophilized from PBS pH 7.4** and shipped as lyophilized powder at ambient temperature. The lyophilized reagent can be stored at 2-8°C for up to 12 months.

After reconstitution in 250 µL, the final concentration of DBCO-conjugated sdAb is 1 mg/mL. For optimal performance, store the reconstituted reagent in aliquots at -80°C.

### Protocol: Reconstitution of DBCO-conjugated sdAbs

1. Prepare sterile 50% glycerol (v/v) in deionized water.  
**Do NOT add sodium azide as a preservative as azides will interfere with click chemistry.**
2. Open the vial containing the lyophilized DBCO-conjugated sdAb.
3. Add 250 µL of sterile 50% glycerol (v/v) in deionized water.
4. Mix gently and allow to sit at room temperature for ~5 min.
5. Optional: Briefly spin down the vial for 2 min at 100 xg using a 50 mL conical tube with tissue paper at the bottom.
6. Distribute into aliquots. Use small tubes and avoid aliquots below 20 µL.
7. Store the reconstituted reagent at -80°C for up to 6 months.

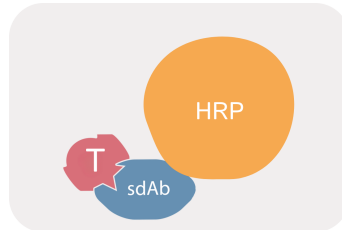
Note: 

- Avoid repeated freeze-thaw cycles.

**Only for research applications, not for diagnostic or therapeutic use!**

### HRP-conjugated sdAbs

HRP-conjugated sdAbs are alpaca-derived single-domain antibodies (sdAbs) conjugated to horseradish peroxidase (HRP) at a 1:1 ratio. These target-specific sdAbs can be applied across various methods (e.g., immunohistochemistry, Western blot, ELISA) using different chemiluminescent or colorimetric substrates.



#### Sketch of an HRP-conjugated sdAb in complex with its target protein.

**T:** target protein

**sdAb:** single-domain antibody

**HRP:** horseradish peroxidase

HRP-conjugated sdAbs are shipped as lyophilized powder at ambient temperature. The lyophilized reagent can be stored at 2-8°C for up to 12 months. Before usage, reconstitute and aliquot the reagent according to the detailed protocol below.

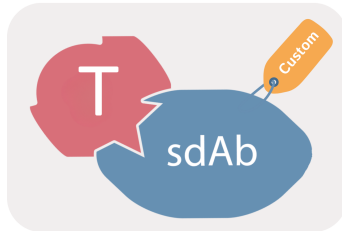
#### Protocol: Reconstitution of HRP-conjugated sdAbs

1. Prepare sterile 50% glycerol (v/v) in deionized water.  
**Do NOT add sodium azide as a preservative as azides will interfere with the activity of HRP.**
  2. Open the vial containing the lyophilized HRP-conjugated sdAb.
  3. Add 200 µL of sterile 50% glycerol (v/v) in deionized water.
  4. Mix gently and allow to sit at room temperature for ~5 min.
  5. Optional: Briefly spin down the vial for 2 min at 100 xg using a 50 mL conical tube with tissue paper at the bottom.
  6. Distribute into aliquots. Use small tubes and avoid aliquots below 20 µL.
  7. Storage:
    - Working aliquot can be stored at 4°C for up to 6 months.
    - For even longer storage, we recommend keeping aliquots at -80°C.
- Note:
- Avoid repeated freeze-thaw cycles.

**Only for research applications, not for diagnostic or therapeutic use!**

### Unconjugated sdAbs

Unconjugated sdAbs are alpaca-derived single-domain antibodies (sdAbs) that generally feature a single ectopic cysteine at the C-terminus. This allows for site-specific conjugation according to the user's preference. These target-specific sdAbs are intended for custom applications requiring direct conjugation to a particular fluorophore, DNA oligonucleotides, or other compounds.



#### Sketch of a custom-conjugated sdAb in complex with its target protein.

**T:** target protein

**sdAb:** single-domain antibody

**Custom:** custom label coupled to an ectopic cysteine

Unconjugated sdAbs featuring an ectopic cysteine are **lyophilized from 5 mM KPi pH 6.0, 300 mM NaCl, 0.5 mM EDTA pH 6.0** and shipped as lyophilized powder at ambient temperature. The lyophilized reagent can be stored at 2-8°C for up to 12 months. Ideally, the reagent should be reconstituted immediately before use according to the standard protocol A.

After reconstitution in 250 µL, the final concentration of unconjugated sdAb is 1 mg/mL.

#### A. Standard reconstitution protocol for immediate use

Note: It is highly recommended to follow this standard protocol for reconstitution and to perform the conjugation reaction immediately after reconstituting the reagent.

1. Open the vial containing the lyophilized unconjugated sdAb.
2. Add 250 µL of deionized water.
3. Mix gently and allow to sit at room temperature for ~5 min.
4. Optional: Briefly spin down the vial for 2 min at 100 *xg* using a 50 mL conical tube with tissue paper at the bottom.
5. Immediately proceed to the custom conjugation reaction.  
**Do not freeze. Freezing and/or storage may result in loss of reactivity.**

#### B. Alternative reconstitution protocol for further storage

Note: **Storage after reconstitution is not recommended** as it may result in loss of reactivity of the ectopic cysteine. **NanoTag does not guarantee the performance of reagents stored after reconstitution.**

1. Open the vial containing the lyophilized unconjugated sdAb.
2. Add 250 µL of sterile 50% glycerol (v/v) in deionized water.
3. Mix gently and allow to sit at room temperature for ~5 min.
4. Optional: Briefly spin down the vial for 2 min at 100 *xg* using a 50 mL conical tube with tissue paper at the bottom.
5. Aliquot, overlay with argon and store at -80°C. Avoid freeze-thaw cycles.

**Only for research applications, not for diagnostic or therapeutic use!**