Fab Antibody Coupling to Bivalent BiCatchers

Protocol

Abstract

This protocol provides the steps to follow to couple a recombinant Fab antibody incorporating a SpyTag2 (Fab), e.g., format Fab-F-Spy2-H, to a bivalent BiSpyCatcher2 and its derivatives (BiCatcher). These guidelines can also be applied to couple a protein with a reactive SpyTag to a BiCatcher.

Short Protocol

1. Calculate the required volumes of Fab and BiCatcher, starting with the amount of Fab you want to couple (see detailed protocol below for calculations).
2. Mix Fab and BiCatcher.
3. Incubate for 1 hr at RT.

Detailed Method

1. To ensure full bivalency of the final coupled Fab and BiCatcher, it is recommended to start with a 25% molar excess of Fab over SpyCatcher sites. Since each BiCatcher contains two SpyCatcher sites, this equals a 2.5 molar excess of Fab e.g., 1 nmol BiSpyCatcher2 + 2.5 nmol Fab-F-Spy2-H. It is possible to use a 2:1 coupling ratio for Fab:BiCatcher (equals a 1:1 ratio for Fab:SpyCatcher sites), but inaccuracies in protein concentration determination might lead to deviations from this ratio and to unpredictable amounts of uncoupled Fab or BiCatcher.
2. When coupling Fab and BiCatcher in solution, it is recommended to use the BiCatcher at the original concentration supplied, and to adjust the concentration of the Fab to 1 mg/ml if practical. [Note 1, 2]
3. Add the required volume of BiCatcher to the Fab. Mix and incubate for 1 hr at RT. It is not important to stop the reaction after 1 hr, it can be left overnight if desired. [Note 3] Assuming the BiCatcher is at the original concentration, if the Fab concentration is 1 mg/ml, the volume of BiCatcher required is 1/10th the volume of Fab, i.e. add 10 µl BiCatcher to 100 µl of Fab; if the Fab concentration is 0.5 mg/ml, the volume of BiCatcher required is 1/20th the volume of Fab, i.e. add 5 µl of BiCatcher to 100 µl Fab.

Note 1: There is no minimum concentration required for coupling, but the coupling reaction is faster when the components are at a higher concentration; the lower the concentration, the slower the reaction.

Note 2: When working with diluted Fab or BiCatcher e.g., immobilized BiCatcher on a resin or ELISA plate, and antibody concentrations in the single- or double-digit µg/ml range, reaction times for complete coupling will be longer and must be determined experimentally.

Note 3: This method can be used for coupling BiCatchers to SpyTag1, SpyTag2, and SpyTag3. A longer reaction time is required when coupling to SpyTag1.

To calculate the required volume of BiCatcher when starting with quantities or concentrations different from above:

$$V(Fab) = \frac{m(Fab)}{conc(Fab)}$$

$$V(BiCatcher) = \frac{m(Fab) * 1{,}000{,}000}{Mw(Fab) * c(BiCatcher) * Valency * Ratio}$$

V(Fab):	Volume of Fab (µl)
m(Fab):	Amount of Fab (µg)
conc(Fab):	Concentration of Fab (mg/ml)
V(BiCatcher):	Volume of BiCatcher (µl)
Mw(Fab):	Molecular weight of Fab (g/mol)
c(BiCatcher):	Molar concentration of BiCatcher (µM)
Valency:	Number of Catcher sites, 2 per BiCatcher
Ratio:	Ratio of Fab:Catcher; 1.25 is recommended for bivalent BiCatchers

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Note 4: Though all Catcher sites should have reacted with the more abundant SpyTagged Fab, it is still possible that some uncoupled SpyCatcher sites remain. As an option, these can be blocked by addition of a 5-fold molar excess of SpyTag3 Peptide (catalog #BLP086) over SpyCatcher followed by incubation for 5 minutes at RT. This step is especially recommended for assays that contain more than one SpyTagged antibody, e.g., sandwich ELISA or multiplex assays.

Quality Control

The success of the reaction can be checked using nonreducing SDS PAGE with Coomassie staining. Run 1–1.5 µg of the coupled product. For comparison, also run the uncoupled Fab and BiCatcher on the same gel.

Recommended Storage

For short term use store aliquots at 2-8°C; for long term storage refer to the conditions recommended on the datasheet for each specific BiCatcher. Avoid repeated freeze-thaw cycles. The addition of 0.0095% methylisothiazolinone (MIT) as a preservative is recommended for storage for up to one month at 2-8 °C.

Calculating the Molar Concentration of the Coupled Antibody

$$c(\text{Product}) = \frac{n(\text{BiCatcher})}{V(\text{BiCatcher}) + V(\text{Fab})} = \frac{c(\text{BiCatcher}) * V(\text{BiCatcher})}{V(\text{BiCatcher}) + V(\text{Fab})}$$

c(Product): Molar concentration of coupled antibody (µM)
n(BiCatcher): Molar amount of BiCatcher used for the reaction (pmol)

To convert the molar concentration to weight concentration:

conc = c(Product) * Mw(Product)

Mw(Product) = Mw(BiCatcher) + 2 * Mw(Fab)

Mw(BiCatcher): see table 1

Mw(Fab): ~ 54,000 g/mol

Table 1. BiCatcher products.

Product	Description	Molecular Weight (Da, calculated)	Catalog Number
BiSpyCatcher2	BiSpyCatcher2 protein	29,018	TZC002
BiSpyCatcher2-CYS	BiSpyCatcher2 with an engineered cysteine residue; can be used for site-specific chemical conjugation to a label of choice[1]	29,235	TZC002CYS
BiSpyCatcher2-CYS3	BiSpyCatcher2 with three engineered cysteine residues; can be used for site-specific chemical conjugation to a label of choice[2]	29,469	TZC002CYS3
BiSpyCatcher2-Biotin	BiSpyCatcher2 with three engineered cysteine residues conjugated to biotin	29,469*	TZC002B
BiSpyCatcher2:HRP	BiSpyCatcher2 with three engineered cysteine residues conjugated to HRP	29,469*	TZC002P

1. BiSpyCatcher2-CYS can dimerize during storage by formation of a disulfide bond via the free cysteines. Before conjugation to these cysteines, the BiSpyCatcher2-CYS dimers should be mildly reduced e.g., by addition of 5 mM DTT and incubation for 1 hr at room temperature. This should be followed by a fast DTT removal step, e.g., by size exclusion chromatography using PD10 columns, as DTT can interfere with the subsequent conjugation chemistry.

2. BiSpyCatcher2-CYS3 contains DTT to avoid oligomerization through disulfide bond formation. Immediately before conjugation, to avoid oxidation and disulfide bond formation, carry out a fast DTT removal step, e.g., by size exclusion chromatography using PD10 columns, as DTT can interfere with the subsequent conjugation chemistry.

Dialysis is not recommended for DTT removal, as the cysteines can oxidize again during this slow process.

*Molecular weight without conjugate

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