Fab Antibody Coupling to Monovalent SpyCatchers

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 monovalent SpyCatcher2 or SpyCatcher3 and its derivatives (SpyCatcher). These guidelines can also be applied to couple a protein with a reactive SpyTag to a monovalent SpyCatcher.

Short Protocol

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

Detailed Method

1. To ensure all Fab is coupled to the SpyCatcher, it is recommended to start with a 25% molar excess of SpyCatcher over Fab. For example, 1 nmol Fab-F-Spy2-H + 1.25 nmol SpyCatcher2. It is possible to use a 1:1 coupling ratio but inaccuracies in protein concentration determination might lead to deviations from this ratio and to unpredictable amounts of uncoupled Fab or SpyCatcher.
2. When coupling Fab and SpyCatcher in solution, it is recommended to use the SpyCatcher at the original concentration supplied, and to adjust the concentration of the Fab to 1 mg/ml if practical. [Note 1, 2]

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 SpyCatcher e.g., immobilized SpyCatcher 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.

3. Add the required volume of SpyCatcher 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 SpyCatcher is at the original concentration, if the Fab concentration is 1 mg/ml, the volume of SpyCatcher required is 1/10th the volume of Fab, i.e. add 10 µl SpyCatcher to 100 µl of Fab; if the Fab concentration is 0.5 mg/ml, the volume of SpyCatcher required is 1/20th the volume of Fab, i.e. add 5 µl of SpyCatcher to 100 µl Fab.

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

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

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

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

V(Fab):	Volume of Fab (µl)
m(Fab):	Amount of Fab (µg)
conc(Fab):	Concentration of Fab (mg/ml)
V(SpyCatcher):	Volume of SpyCatcher (µl)
Mw(Fab):	Molecular weight of Fab (g/mol)
c(SpyCatcher):	Molar concentration of SpyCatcher (µM)
Valency:	Number of Catcher sites, 1 per SpyCatcher
Ratio:	Ratio of Fab:Catcher; 0.8 is recommended for monovalent SpyCatchers

BIO-RAD

Note 4: When an excess of SpyCatcher is used, there will be uncoupled SpyCatcher remaining after completion of the protein ligation reaction. It is recommended to block the SpyCatcher site by addition of a 2.5-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 SpyCatcher 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 SpyCatcher. 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

$$c(\text{Product}) = \frac{n(\text{Fab})}{V(\text{Fab}) + V(\text{SpyCatcher})} = \frac{m(\text{Fab})}{Mw(\text{Fab})\,(V(\text{Fab}) + V(\text{SpyCatcher}))}$$

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

To convert the molar concentration to weight concentration:
conc = c(Product) ∗ Mw(Product)

Mw(Product) = Mw(Fab) + Mw(SpyCacther)

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

Mw(SpyCatcher): see table 1

Table 1. SpyCatcher products.

Product	Description	Molecular Weight (Da, calculated)	Catalog Number
SpyCatcher2	SpyCatcher2 protein	15,687	TZC001
SpyCatcher2-CYS	SpyCatcher2 with an engineered cysteine residue; can be used for site-specific chemical conjugation to a label of choice[1]	15,904	TZC001CYS
SpyCatcher2:Biotin	SpyCatcher2 with an engineered cysteine residue conjugated to biotin	15,904*	TZC001B
SpyCatcher3	SpyCatcher3 protein	15,151	TZC025
SpyCatcher3-CYS	SpyCatcher3 with an engineered cysteine residue; can be used for site-specific chemical conjugation to a label of choice[1]	15,368	TZC025CYS

1. SpyCatcher2-CYS and SpyCatcher3-CYS can dimerize during storage by formation of a disulfide bond via the free cysteines. Before conjugation to these cysteines, the SpyCatcher2-CYS or SpyCatcher3-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 desalting 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.

Visit **bio-rad-antibodies.com/spycatcher** for further information.