

Fab Antibody Coupling to Bivalent FcCatchers 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 FcSpyCatcher3 and its derivatives (FcCatcher). These guidelines can also be applied to couple a protein with a reactive SpyTag to an FcCatcher.

Short Protocol

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

Detailed Method

1. To ensure full bivalency of the final coupled Fab and FcCatcher, it is recommended to start with a 25% molar excess of Fab over SpyCatcher sites. Since each FcCatcher contains two SpyCatcher sites, this equals a 2.5 molar excess of Fab e.g., 1 nmol FcSpyCatcher3 + 2.5 nmol Fab-F-Spy2-H. It is possible to use a 2:1 coupling ratio for Fab:FcCatcher (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 FcCatcher.
2. When coupling Fab and FcCatcher in solution, it is recommended to use the FcCatcher 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 dilute Fab or FcCatcher e.g., immobilized FcCatcher 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 FcCatcher 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 FcCatcher is at the original concentration, if the Fab concentration is 1 mg/ml, the volume of FcCatcher required is 1/10th the volume of Fab, i.e. add 10 µl FcCatcher to 100 µl of Fab; if the Fab concentration is 0.5 mg/ml, the volume of FcCatcher required is 1/20th the volume of Fab, i.e. add 5 µl of FcCatcher to 100 µl Fab.

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

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

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

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

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

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 FcCatcher 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 FcCatcher. 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

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

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

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

$$Mw(\text{Product}) = Mw(\text{FcCatcher}) + 2 * Mw(\text{Fab})$$

Mw(FcCatcher): see table below

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

Table 1. FcCatcher products.

Product	Description	Molecular Weight (Da, calculated)	Catalog Number
hIgG1-FcSpyCatcher3	SpyCatcher3 fused to the hinge region, CH2, and CH3 of human IgG1	77,166	TZC009
hIgG1-FcSpyCatcher3:Biotin	SpyCatcher3 fused to the hinge region, CH2, and CH3 of human IgG1 and conjugated to biotin	77,166*	TZC009B
hIgG2-FcSpyCatcher3	SpyCatcher3 fused to the hinge region, CH2, and CH3 of human IgG2	76,372	TZC016
hIgG3-FcSpyCatcher3	SpyCatcher3 fused to the hinge region, CH2, and CH3 of human IgG3	87,394	TZC017
hIgG4-FcSpyCatcher3	SpyCatcher3 fused to the hinge region, CH2, and CH3 of human IgG4	76,494	TZC018
hIgG4-Pro-FcSpyCatcher3	SpyCatcher3 fused to the hinge region, CH2, and CH3 of human IgG4-Pro (S228P)	76,514	TZC019
hIgA-FcSpyCatcher3	SpyCatcher3 fused to the hinge region, CH2, and CH3 of human IgA	79,656	TZC020
mIgG2a-FcSpyCatcher3	SpyCatcher3 fused to the hinge region, CH2, and CH3 of mouse IgG2a	77,738	TZC012
rbIgG-FcSpyCatcher3	SpyCatcher3 fused to the hinge region, CH2, and CH3 of rabbit IgG	76,132	TZC013

*Molecular weight without conjugate.

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

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