NovaFluor™ Conjugation Kit

Pub. No. MAN0025061 Rev. G

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. SDSs are available from thermofisher.com/support.

Product description

The Invitrogen™ NovaFluor™ Conjugation Kit provides a simple workflow to conjugate an ultraviolet (UV), violet, blue, yellow-green, or red excitable fluorophore to your antibody of choice in just 3 easy steps. NovaFluor™ dyes have unique spectral signatures designed with narrow emission, minimal cross-laser excitation, and decreased spillover-spread for higher panel resolution. NovaFluor™ dyes have been designed to emit at unique wavelengths, so they can be used to replace traditional fluorophores or to fill in the spectral spaces with otherwise unused detectors, allowing for the easy addition of extra markers in your panel.

Contents and storage

Table 1 NovaFluor™ Conjugation Kit

Contents	Quantity	Storage
NovaFluor™ label[1]	1 each	4°C
NovaFluor™ Linker	1 each	–20°C
Conjugate Stabilization Buffer	1 each	4°C
CellBlox™ Plus Blocking Buffer	2 each	4°C
Zeba™ Spin Desalting Columns, 7K MWCO	2 each	4°C
Capless collection tubes	2 each	4°C
Activation Reagent	1 each	–20°C
Reaction Buffer	2 each	4°C
PBS	1 each	4°C
Ammonium Sulfate	1 each	Room temperature

[1] See Table 2.

Table 2 Available NovaFluor™ conjugation kits

Conjugate kit	Cat. No.
NovaFluor™ Blue 510 Conjugation Kit	K06T04L008
NovaFluor™ Blue 530 Conjugation Kit	K06T04L002
NovaFluor™ Blue 555 Conjugation Kit	K06T04L009
NovaFluor™ Blue 585 Conjugation Kit	K06T04L010
NovaFluor™ Blue 610-30S Conjugation Kit	K06T04L003
NovaFluor™ Blue 610-70S Conjugation Kit	K06T04L011
NovaFluor™ Blue 660-40S Conjugation Kit	K06T04L004
NovaFluor™ Blue 660-120S Conjugation Kit	K06T04L012
NovaFluor™ Blue 690 Conjugation Kit	K06T04L021

Conjugate kit	Cat. No.
NovaFluor™ Blue 725 Conjugation Kit	K06T04L033
NovaFluor™ Blue 745 Conjugation Kit	K06T04L034
NovaFluor™ Blue 760 Conjugation Kit	K06T04L026
NovaFluor™ Blue 800 Conjugation Kit	K06T04L027
NovaFluor™ Yellow 570 Conjugation Kit	K06T04L005
NovaFluor™ Yellow 590 Conjugation Kit	K06T04L013
NovaFluor™ Yellow 610 Conjugation Kit	K06T04L006
NovaFluor™ Yellow 660 Conjugation Kit	K06T04L007
NovaFluor™ Yellow 690 Conjugation Kit	K06T04L014
NovaFluor™ Yellow 700 Conjugation Kit	K06T04L015
NovaFluor™ Yellow 730 Conjugation Kit	K06T04L016
NovaFluor™ Yellow 755 Conjugation Kit	K06T04L022
NovaFluor™ Yellow 810 Conjugation Kit	K06T04L028
NovaFluor™ Red 660 Conjugation Kit	K06T04L017
NovaFluor™ Red 685 Conjugation Kit	K06T04L018
NovaFluor™ Red 700 Conjugation Kit	K06T04L019
NovaFluor™ Red 710 Conjugation Kit	K06T04L020
NovaFluor™ Red 725 Conjugation Kit	K06T04L023
NovaFluor™ Red 755 Conjugation Kit	K06T04L024
NovaFluor™ Red 800 Conjugation Kit	K06T04L035
NovaFluor™ Violet 690 Conjugation Kit	K06T04L030
NovaFluor™ Violet 745 Conjugation Kit	K06T04L031
NovaFluor™ Violet 800 Conjugation Kit	K06T04L029
NovaFluor™ Ultraviolet 765 Conjugation Kit	K06T04L032

Procedural guidelines

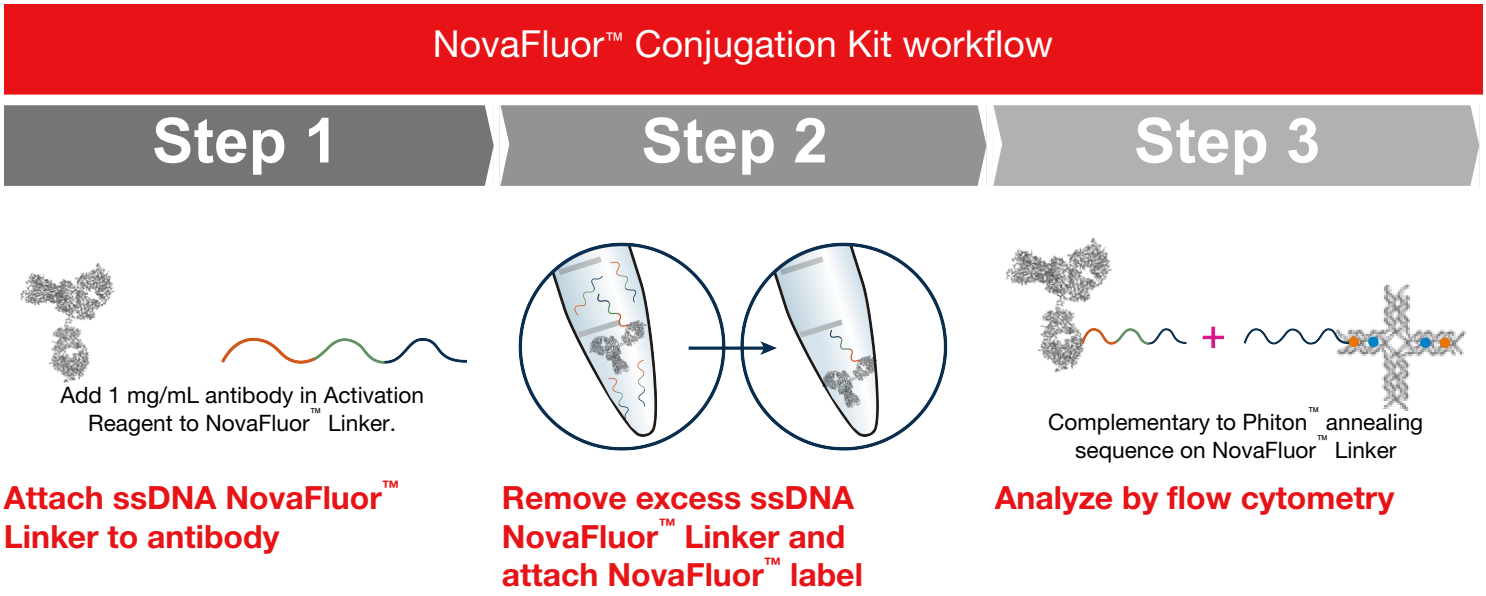
- Read all steps before you begin, to familiarize yourself with the procedure.
- Check that your antibody is compatible with this kit. Your antibody should be pure (free of secondary proteins that will interfere with labeling) and at a concentration of 1 mg/mL. If your concentration is lower than 1 mg/mL, we recommend use of a 30K MWCO protein concentrator (e.g., Pierce™ Protein Concentrators, PES, Cat. No. 88502) to quickly concentrate your antibody.
- The buffer your antibody is stored in is not important, as you will buffer-exchange your antibody into our optimized Reaction Buffer.
- Use the CellBlox™ Plus Blocking Buffer whenever staining cells with NovaFluor™ conjugates, including single-color controls using cells. Use of CellBlox™ Plus Blocking Buffer requires minimal change to most flow cytometry staining protocols.

Before you begin

If your antibody is >1 mg/mL, dilute it to 1 mg/mL prior to beginning the protocol.

Workflow

NovaFluor™ Conjugation Kit provides a rapid and simple means to conjugate oligos to antibodies. The subsequent clean-up removes excess free DNA and allows you to add the NovaFluor™ conjugate of your choice to an antibody with minimal effort or antibody loss.



Antibody and buffer requirements for NovaFluor™ Conjugation Kit

Table 3 Antibody and buffer requirements

Starting antibody/buffer requirements	Compatibility
≥1 mg/mL antibody	Minimum requirement (see “Before you begin” on page 2)
BSA or other stabilizing proteins	Not recommended as these will be labeled as well
Compatible buffers	Any buffer

Attach NovaFluor™ Linker to antibody

1. Remove Activation Reagent and NovaFluor™ Linker from -20°C and allow to come to room temperature. Remove the Zeba™ spin column from the 2 mL collection tube, invert 2–3 times. Twist and snap off the bottom.

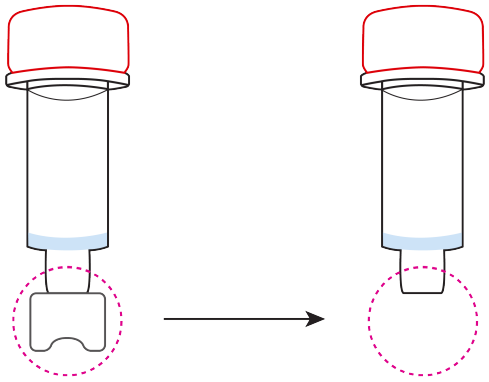


Figure 1 Spin column preparation

2. Slightly loosen the caps on the spin columns and place the spin columns back into the 2 mL collection tubes. On each spin column, make a mark on the plastic rim just below the cap.

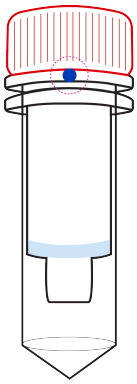


Figure 2 Spin column with mark

Do not to mark the collection tube or the cap of the spin column. Label the spin columns “1” and “2”.

3. For all centrifugation steps, orient the spin columns with the mark on the rim facing out.

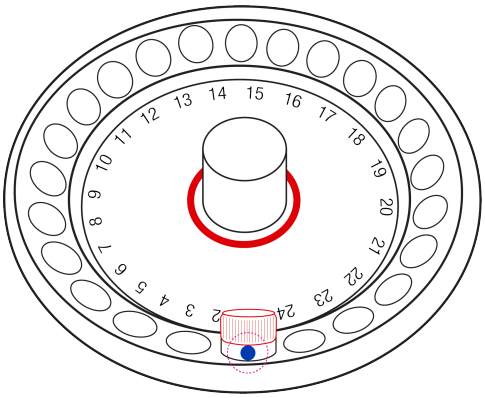


Figure 3 Spin column orientation The spin column should be within the collection tube and aligned with the dot facing outward. This should be the orientation for all centrifugation steps. The orientation of the collection tube is not important.

4. Centrifuge at 1,500 × *g* for 1 minute.

   After centrifugation, the resin bed should angle up toward the mark made on the rim.

5. Remove both spin columns from the collection tubes and discard the eluent. Place the spin columns back into the collection tubes.

6. Remove the caps from the spin columns and add 300 µL of Reaction Buffer to each spin column.

7. Replace the caps and screw on slightly (do not seal). Centrifuge at 1,500 × *g* for 1 minute.

8. Repeat step 5–step 7 three times for a total of four buffer exchanges.

9. After the fourth buffer exchange, remove spin column 2 from the collection tube and discard the eluent.

10. Add 300 µL of Reaction Buffer to spin column 2 and cap tightly. Set spin column 2 aside.

11. Transfer spin column 1 to a clean 1.5 mL microcentrifuge tube. Add your antibody solution (100 µL, 1 mg/mL) to spin column 1. Replace the cap (do not seal) and centrifuge at 1,500 × *g* for 2 minutes.

12. Dispose of spin column 1.

    Your antibody is now buffer exchanged and is ready to be activated.

13. Add the antibody solution from the 1.5 mL microcentrifuge tube from step 11 into the tube of Activation Reagent.

14. Pipet up and down 3–4 times to begin to dissolve the Activation Reagent.

15. Cap the tube tightly, then vigorously vortex for 3–4 seconds or sharply invert the tube 3–4 times to mix the solution.

16. Continue to briefly mix the solution 2–3 more times over the course of 1 minute. Then, centrifuge at 1,500 × *g* for 30 seconds to collect the solution at the bottom of the tube.

17. Let the solution incubate for 1 hour at room temperature. There is no need to continue to mix the solution during this incubation period.

IMPORTANT! The timing of step 17 is critical to the performance of your final conjugate. In our experience, all antibodies tested perform well using a 1-hour activation. After 3 hours, some antibodies exhibit a marked improvement in brightness, while others are completely inactivated.

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18. After 1 hour, loosen the cap on spin column 2 (it should still be in the 2 mL collection tube). Centrifuge at 1,500 × *g* for 1 minute.

19. Discard the eluent and transfer spin column 2 to a fresh 1.5 mL microcentrifuge tube.

20. Add all of the antibody solution from the tube of Activation Reagent to spin column 2. Centrifuge at 1,500 × *g* for 2 minutes.

21. Remove spin column 2 from the microcentrifuge tube and dispose of spin column 2.

22. Transfer the activated antibody solution from the 1.5 mL microcentrifuge tube from step 21 into the tube of NovaFluor™ Linker. Cap tightly, then vigorously vortex for 5–10 seconds or invert sharply 5–10 times to mix.

23. Centrifuge at 1,500 × *g* for 30 seconds to collect the solution at the bottom of the tube.

24. Incubate for 2 hours at room temperature.

    Note: The sample can be incubated up to overnight with no loss in performance of the final conjugate.

Remove excess ssDNA linker and attach NovaFluor™ label

1. Add 80 µL of PBS to the antibody solution and mix by pipetting up and down 3–4 times.

2. Add 200 µL of ammonium sulfate to the antibody solution and mix by gently pipetting up and down 10 times. Incubate on ice for 10 minutes.

   Note: After receiving the ammonium sulfate solution, it is possible that some crystals may have formed in the saturated solution. This is to be expected. Prior to use, it is recommended that the ammonium sulfate be warmed to 37°C for 15 minutes, then vortexed and spun down.

3. Centrifuge at 13,500 x *g* for 5 minutes. Your antibody should clearly precipitate from solution as a white pellet. Carefully draw off the supernatant with a P200 pipette, making sure not to disturb the pellet. Dispose of the supernatant.

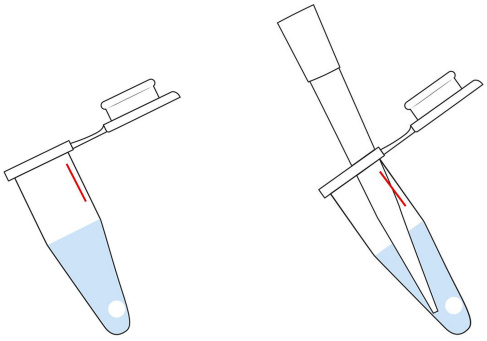

Figure 4 Antibody and supernatant removal

4. Resuspend the pellet into 200 µL of PBS.

   Note: It is easiest to do this by pipetting up and down slowly with the tip touching the bottom of the tube, which should help to break up the pellet. It may take a few minutes for your antibody to fully dissolve.

5. Repeat step 2 and step 3.

6. Resuspend the pellet into 100 µL of PBS. See note, step 4.

7. Add 300 µL of the desired NovaFluor™ Label to the antibody solution and vortex for 5–10 seconds or invert 5–10 times to mix.

8. Centrifuge at 1,500 x *g* for 30 seconds to collect the solution at the bottom of the tube.

9. Incubate at 4°C for 1 hour protected from light.

10. Stabilize this final conjugate product with the addition of 5X NovaFluor™ Stabilization Buffer. Add 100 µL of the provided 5X NovaFluor™ Stabilization Buffer to 400 µL of the antibody conjugate and mix well. The antibody is now labeled and stabilized at an approximate concentration of 180 µg/mL or 1.2 µM.

    Note: Store your NovaFluor™ conjugated antibody at 4°C. If the antibody is to be diluted further, we recommend doing so with a 1X solution of 5X Conjugate Stabilization Buffer, prepared by diluting 1:4 with PBS.

11. Add 5 µL of CellBlox™ Plus Blocking Buffer directly to a cell suspension containing 10^3–10^8 cells prior to the addition of an antibody, with 100 µL as a final staining volume.

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0025061 G

Revision	Date	Description
G	8 October 2025	New product SKUs were added to the manual and minor edits.
F	18 September 2024	New product SKUs were added to the manual and minor edits.
E.0	23 May 2023	The user manual was updated to include additional products.
D.0	15 November 2022	New product SKUs were added to the manual.
C.0	7 September 2022	The user manual was updated to include an additional product.
B.0	14 March 2022	The user manual was updated to include additional products, edited product description, procedural guidelines, recommendations for use of blocking buffer, and a revision history table.
A.0	20 April 2021	New manual for the NovaFluor™ Conjugation Kit.