MANUAL

Oligonucleotide Antibody Conjugation Kit (Amine)

Catalog #: OAC-AMINE-001, OAC-AMINE-005

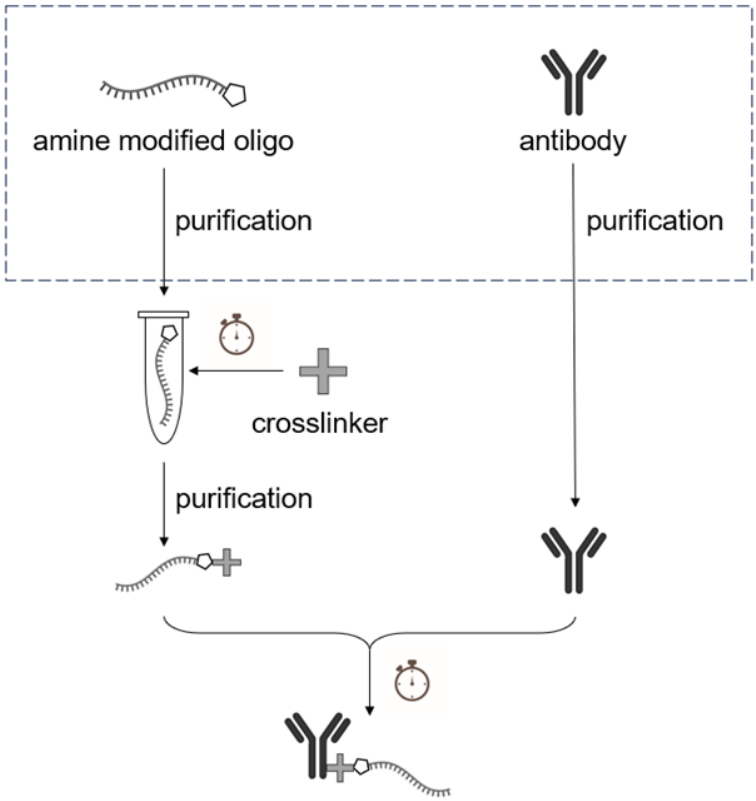
ISO 13485

Introduction

Oligo-antibody conjugates have been widely used in research to serve as oligo-delivery tools or detection methods. RayBio® Oligonucleotide Conjugation Kit provides a simple and highly efficient method to generate oligo-antibody conjugates with less than 20 minutes of on-hand time. This kit can be used to generate the conjugates of different oligo:antibody ratios and achieve up to 90% or higher conjugation efficiency (percentage of antibody conjugated with at least one oligo).

The oligos should be synthesized to include a terminal amine group at either 5' or 3' end and purified by HPLC to remove any impurities that may affect the conjugation. The antibody intended for conjugation should also be purified and must be free from BSA, Tris-Glycine, or any other amine-containing impurities, as these can substantially impact the conjugation process. Each reaction is designed to conjugate 100 µg of antibody and a control. This kit contains enough reagents to run 2 reactions (-001) or 10 reactions (-005).

As expected for any chemical conjugation reaction, the concentration and buffer formulation of the oligo and the antibody need to fall within certain parameters, as detailed in this booklet. A single-strand oligo (68 nt) and a monoclonal antibody are also provided as positive controls.



Note: *The circled steps for purifying the oligo and antibody should be prepared before the utilization of this conjugation kit to ensure a successful and highly efficient reaction. For antibodies, dialysis, or buffer exchange through desalting can be used to remove the amine-containing impurities.*

Storage

Upon receipt, store the vial of crosslinker, control antibody, control oligo, and desalting column at 4 ºC and other kit components at room temperature.

Component	Size / Description		Storage	Storage After Preparation
	-001	-005		
Conjugation Buffer	1 vial (5 ml)	1 vial (15 ml)	RT	RT
Desalt Spin Column	3 columns	11 columns	4 ºC	4 ºC
Crosslinker Reagent	2 vials	10 vials	4 ºC	-20 ºC
Crosslinker Solvent	1 vial	1 vial	RT	RT
Control antibody (lyophilized)	1 vial (30 µg)	1 vial (30 ug)	4 ºC	4 ºC
Control oligo (lyophilized)	1 vial (6 nmole)	1 vial (6 nmole)	4 ºC	Prepare immediately before use. Any unused oligo may be stored at -20 ºC

RT = room temperature

Note: once resuspended, the control oligo, crosslinker should be stored at -20 ºC, while the control antibody should be stored at 4 ºC. Each vial of the Crosslinker Reagent is enough for conjugating 100 µg desired antibody and 30 µg Control Antibody at the same time.

Additional Materials Required

1. Precision pipettes to deliver 2 µl to 1 ml
2. Nuclease-free barrier filter tips fitting the pipettes. Elongated 10 µl tips are recommended to prevent contamination when processing multiple oligos
3. Nuclease-free water
4. Nuclease-free 1.5-2 ml microcentrifuge tubes
5. Terminal amine-modified oligonucleotides for conjugation (HPLC grade)
6. Antibodies for conjugation
7. Vortex
8. Variable-speed benchtop microcentrifuge

Assay Procedure

1. Calculation

Note: an oligo:antibody ratio of 10:1 ensures most antibodies are conjugated with at least one oligo. However, the kit can be used to generate oligo-antibody conjugates with a range of oligo:antibody ratios. A low oligo:antibody ratio may lead to low conjugation efficiency, but the conjugate may be more homogenous. In contrast, a high oligo:antibody ratio allows for more oligos to be conjugated to the antibody, but over-conjugation may lead to loss of activity of the antibody and high oligo residue in the final product. The best ratio will depend on the purpose of using the conjugate and may be determined experimentally.

1.1. Calculate the amount of oligo needed based on the amount of antibody used.

$$\text{ml antibody} \times \frac{\text{mg antibody}}{\text{1 ml antibody}} \times \frac{\text{mmol antibody}}{\text{1 mg antibody}} \times \text{oligo:antibody ratio} = \text{mmol oligo}$$

Note: an oligo:antibody ratio of 10 is recommended for general application.

1.2. Calculate the volume of 200 µM oligo solution to activate.

$$\frac{\text{mmol oligo}}{\text{200 µM}} \times 1000 = \text{µl oligo}$$

Note: 200 µM is the concentration of oligo stock prepared in step 2.1.

2. Oligo activation

2.1. Resuspend amine-modified oligo powder in Conjugation Buffer to a final concentration of 200 µM.
Note: if the oligo solution was dissolved in another buffer already, use ethanol precipitation to remove the buffer and resuspend the oligo pellet in the Conjugation buffer.
2.2. To prepare the Crosslinker Reagent, add 50 µl Crosslinker Solvent to the Crosslinker Reagent vial. Vortex until fully dissolved. Before use, ensure that the prepared Crosslinker Reagent is brought to room temperature.
2.3. Activate the purified amine-modified oligo by mixing the volume of the 200 µM oligo solution prepared in the Conjugation Buffer calculated in step 1.2 with half the volume of Crosslinker Reagent prepared in step 2.2 in a clean microcentrifuge tube. Vortex to mix well. Following use, promptly close and store the Crosslinker Reagent at -20°C.
Note: for example, if 20 µl of the oligo solution needs to be activated, add 10 µl of the Crosslinker Reagent. The extra oligo solution can be saved at -20 ºC for future use. The Crosslinker Reagent is not stable and may be stored at -20 ºC for no more than one week.
2.4. Incubate the tube at room temperature for 30 minutes. No rocking is required. During the incubation, prepare the desalting column as described in steps 3.1 to 3.4.

3. Oligo purification

3.1. Take a new desalting column, remove its bottom closure, and loosen the cap. Place the column in a 1.5-2 ml collection tube. Spin the desalting column at 1,500× g for 1 minute to remove the storage solution. Blot the bottom of the column to remove excess liquid.
3.2. Place a mark on the side of the column where the compacted resin is slanted upward. Place the column in the microcentrifuge with the mark facing outward in all subsequent centrifugation steps.
3.3. Add 300 µl Conjugation Buffer on top of the resin bed and spin at 1,500× g for 1 minute. Remove the flowthrough.
3.4. Repeat step 3.3 one more time. Then, transfer the column into a new microcentrifuge tube and leave it at room temperature until use.
3.5. After the 30-minute incubation from step 2.4, transfer the desalted oligo activation mix onto the desalting column, and spin at 1,500× g for 2 minutes. Collect the flow-through containing purified activated oligo.

4. Oligo conjugation to antibody

4.1. Mix the desired amount of activated oligo and purified antibody in a clean microcentrifuge tube. For convenience, the volumes of activated oligo needed for 100 µg antibody at different ratios are listed below.

Antibody/µg	Oligo:antibody ratios	Activated oligo/µl
100	1:1	5
100	1:3	15
100	1:5	25
100	1:10	50
100	1:20	100

Note: the activated oligo is not stable and should be used as soon as possible. Discard any unused activated oligo.
4.3. Incubate the tube overnight at room temperature or 4 ºC for better stability of antibodies. If the use is urgent, the incubation may be shortened to 1 hour with lower conjugation efficiency.
4.4. After incubation, depending on the characteristics of the antibody used, the conjugates may be saved at 4 ºC for short term storage or -20 ºC for longer term. The addition of preservatives may help to maintain stability if they are not interfering with the subsequent experiments.

5. Use of the Control Oligo and Control Antibody

5.1. Resuspend the Control Oligo to 200 µM by adding 30 µl Conjugation Buffer. Resuspend the Control Antibody by adding 30 µl nuclease free water to final concentration 1 mg/ml. The control reagents are ready for conjugation.

5.2. Save a small amount of each control reagent for potential analysis described in Analysis of the Conjugate.

5.3. Perform the conjugation following the same procedure described in part 4 with desired oligo:antibody ratio.

Note: the amount of Control Oligo provided is enough for conjugating the Control Antibody for oligo:antibody ratio up to 20:1.

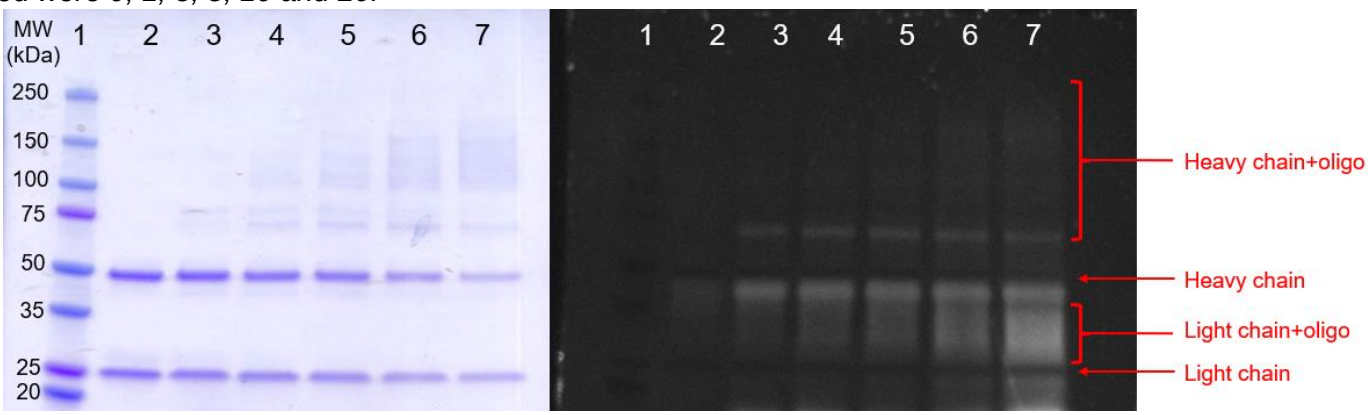
Analysis of the Conjugate

Note: the oligo-antibody conjugates can be analyzed in several ways. The best method to confirm conjugation is a positive result in the experiment in which the conjugate will be used in. Here, gel electrophoresis is used to analyze the presence of conjugates.

6.1. Use a small amount (3-5 µg) of the conjugate to run a routine SDS-PAGE. A 4-12% gradient gel is recommended for best results. Use the same amount of untreated antibody as control.

6.2. Once finishing the electrophoresis, stain the gel for protein using Coomassie Blue stain or a suitable equivalent. After destaining the Coomassie Blue, the gel can be stained for oligo by use SYBR Gold or a suitable equivalent. The overlap of an antibody band and oligo band will confirm the presence of conjugates.[KB1][TW2]

Note: in an SDS-PAGE, the antibody conjugated with oligo will move slower as the molecular weight is increased. Larger oligos will generate a larger band shift and vice versa. Typical SDS-PAGE results of IgG conjugate stained with Coomassie Blue and SYBR Gold are shown. From left to right, the oligo:antibody ratios used were 0, 1, 3, 5, 10 and 20.



Assay Procedure Summary

1. Calculate oligo:antibody ratio (1:10 is standard)
2. Oligo activation
3. Oligo purification
4. Prepare controls
5. Conjugation
6. Analysis of the conjugate
7. Conjugate purification* (if necessary for downstream use)

   **Included in Appendix*

Troubleshooting Guide

Problem	Possible Cause	Solution
Low conjugation efficiency	Amine containing impurities in the oligo or/and antibody	Repeat the purification steps of oligo or/and antibody
	Too low oligo:antibody ratio used	Use higher oligo:antibody ratio
	The activated oligo loses reactivity	Prepare fresh activated oligo and use it at once
	Antibody concentration was too low	Protect samples from light until labeling reagent addition
		Concentrating the antibody to at least 200 µg/ml
Antibody loses activity	Too many oligoes conjugated to the antibody	Use lower oligo:antibody ratio

This product is for research use only.

Appendix- Purification of the Conjugate

Note: the oligo-antibody conjugates can be purified in several ways depending on the tolerance of the downstream experiment. For example, if the oligo used is small, dialysis or desalting can be used. Here, we describe the general protocol purification of conjugates by ion exchange chromatography. A Q column with high resolution is required to separate the conjugates from excess oligo. Adaptation and optimization may be required based on the specific equipment and column used.

7.1. Prepare buffers for the chromatograph. Buffer A: 50 mM Tris-HCl, pH 8.0. Buffer B: 1 M NaCl in 50 mM Tris-HCl, pH 8.0.

7.2. Set the purification program as below. The flow rate should be determined by the equipment and column used. Record the absorbance at 260 and 280 nm on a photometer.

Steps	NaCl concentration/mM	Column volume (CV)
Equilibrate	150	5
Sample application	150	2× of sample volume
Elution	From 150 to 1000	16
Column wash	1000	3
Equilibrate	150	5

7.3. Dilute a small amount of the activated oligo in 15% (v/v) buffer B. Load and analyze the sample based on the program described above to determine the location of the pure oligo peak.

7.4. Dilute the conjugate in 15% (v/v) buffer B. Load and analyze the sample based on the program described above. Collect all of the outflow during the elution step with a fraction of 0.5-1 ml.

Note: the conjugate peak should come out with a comparatively strong salt concentration and be located close to the pure oligo peak.

7.5. Concentrate the fractions containing desired peaks by protein concentrators. Then, use the protein concentrators to do a buffer exchange against PBS to remove the high amount of salt.

7.6. Use a small amount of the concentrated peaks to perform an experiment that the conjugates will be used in. Based on the positive result to select the correct peaks containing conjugates.

7.7. The purified conjugates can be stored at 4 ºC for a short time. For long term storage, preservatives may be added.