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# ab218260 Oligonucleotide Conjugation Kit

## A product of Expedeon, an Abcam company

Applicable to Expedeon product codes: 425-000, 425-0300

For the covalent conjugation of antibodies to oligos.

This product is for research use only and is not intended for diagnostic use.

To get Chinese protocol, please scan QR code via WeChat or visit Protocols section at www.abcam.cn/ab218260

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# 1. Overview

The Oligo Conjugation Kit (ab218260) enables the easy and efficient generation of antibody-oligo conjugates in less than 2 hours. The kit can be used to generate conjugates of different antibody: oligo ratios. It also includes positive controls that allow the user to confirm that the conjugation chemistry has worked correctly.

This kit can be used to conjugate antibodies to single-stranded oligos that are 10-120 bases long or to double-stranded oligos that are up to 80 bases long. The oligos should be synthesized to include a terminal amine group.

The 1-test kit includes reagents for 1 conjugation of 100 µg of antibody and 1 control.

The 3-test kit includes reagents for 3 conjugations of 100 µg of antibody and 1 control.

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.

## 2. Materials Supplied and Storage

Upon receipt, store the Activation Reagents and buffers pack at 4°C and the Conjugate Clean Up Reagent and Separating Columns at room temperature.

| Item | Quantity | | Storage Condition |
|---|---|---|---|
| | 1 x 100 µg | 3 x 100 µg | |
| Oligo Activation Reagent | 2 Vials | 4 Vials | 4°C |
| Antibody Activation Reagent | 2 Vials* | 4 Vials** | 4°C |
| Oligo Control (freeze dried) | 1 Vial | 1 Vial | 4°C |
| Antibody Control (freeze dried) | 1 Vial | 1 Vial | 4°C |
| Separating Columns | 4 Units | 8 Units | RT |
| Wash Buffer | 80 mL | 160 mL | 4°C |
| Conjugate Clean Up Reagent | 2 mL | 6 mL | RT |
| Antibody Suspension Buffer | 0.5 mL | 1 mL | 4°C |

*(1 reaction + 1 control); **(3 reactions + 1 control)

## 3. Technical considerations

### 3.1 Pre-conjugation considerations for the oligo

This kit can be used to conjugate both single- and double-stranded oligos. A single-stranded oligo must be 10-120 bases long and contain a terminal amine group, which must be added during synthesis. (All commercial oligo suppliers offer this modification.) The efficiency of conjugation is slightly higher with 5'aminated oligos. Double-stranded oligos can be up to 80 bases in length but only one end should be aminated.

The oligo must be purified by HPLC, at a concentration of 60-100µM in 100 µL of a suitable buffer (see below). If the oligo concentration is greater than 100 µM, dilute to 100 µM in Wash Buffer.

The reaction volume should not be different from 100 µL.

## 3.2 Pre-conjugation considerations for the antibody

This kit has been designed to conjugate 100 µg of antibody per reaction. For optimal results, the antibody must be purified and at a concentration of 1 mg/mL in 100 µL of a suitable buffer (see below). Higher antibody concentrations should be diluted to 1 mg/mL with Wash Buffer.

It is possible to conjugate 50 µg of antibody per reaction (0.5 mg/ml in 100 µL), however this is the lowest limit for seeing a clearly visible pellet during the purification step. Lower amounts of antibodies are not advised for the conjugation. In this case the antibody should be concentrated prior to conjugation using the Antibody Concentration and Clean-Up Kit (ab102778).

The reaction volume should not be different from 100 µL.

## 3.3 Buffer Considerations

| Buffer component | Oligo buffer | Antibody buffer |
|---|---|---|
| pH | 6-8 | 7-9 |
| Amine free buffer (ideally phosphate buffer) | YES | YES |
| Non-buffering salts (e.g. sodium chloride) | YES | YES |
| Chelating agents (e.g. EDTA) | YES | YES |
| Sugars | YES | YES |
| Glycerol | <50% | <50% |
| Thiomersal (Thimerosal, Methiolate) | NO | NO |
| Sodium Azide* | <0.1% | <0.1% |
| BSA* | <0.1% | <0.1% |
| Gelatin* | <0.1% | <0.1% |
| Tris | NO | <20 mM |
| Glycine | NO | NO |
| Primary amines | NO | NO |
| Thiols (e.g. mercaptoethanol or DTT) | NO | NO |

**ΔNote:** *Individually, the concentrations shown for these components should not affect the reaction. However, in combination with other compounds that are not recommended above a certain concentration, the reaction may be affected.

# 4. Assay Procedure

## 4.1 Oligo Activation

Add 100 µL of the oligo to the Oligo Activation Reagent vial. Mix gently and incubate for 30 minutes at room temperature. Proceed to 4.2 during the incubation.

## 4.2 Antibody Activation

Add 100 µL of the antibody (at 1 mg/mL concentration) to the Antibody Activation Reagent vial. Mix gently and incubate for 30 minutes at room temperature. Proceed to 4.3 during the incubation.

## 4.3 Desalting of Activated Material

*Δ **Note**: Use one column for each sample to be desalted. The columns are designed for single use. Discard after use.*

4.3.1 Secure each Separating Column in a vertical position. Remove the upper cap first. Then remove the lower cap and allow the storage liquid to flow through the column. Discard the column flow-through.

4.3.2 Equilibrate each column by adding 3 mL of Wash Buffer to the top of the column and allowing the liquid to flow through under gravity. Discard the flow-through. Repeat a further 4 times.

4.3.3 After the 30-minute incubation for activation in 4.1 and 4.2, add 100 µL of activated oligo or antibody to the top of the column and allow the liquid to completely absorb into the column. Collect the flow through and keep until you confirmed successful conjugation.

4.3.4 Add 550 µL of Wash Buffer to the top of the column. This will push the activated material to the bottom of the column. Allow this liquid to completely absorb before proceeding to the next step. Collect the flow through and keep until you confirmed successful conjugation.

4.3.5 Place a clean microcentrifuge tube under the column. Add 300 µL of Wash Buffer to the top of the column.

4.3.6 Collect the eluate from the column. This column eluate (300 µL) contains activated oligo or antibody that is ready for use in conjugation.

## 4.4 Storage of Activated Material

### Oligo and Control Oligo

The activated oligo can be stored at room temperature for up to 8 hours. For long-term storage of up to 12 months, storage at -20°C is recommended.

### Antibody and Control antibody

The activated antibody should be stored on ice and used within 2 hours. The activated antibody is not stable enough for long-term storage.

## 4.5 Generation of Purified Antibody-oligo Conjugate

This kit can be used to generate antibody-oligo conjugates with a range of antibody: oligo ratios. Simply add different amounts of oligo to the antibody, as described in the table below. The preferred ratio will depend upon the experiment that the conjugate will be used in and may need to be determined experimentally.

4.5.1 Add 300 µL of activated antibody to the appropriate volume of activated oligo and Wash Buffer as shown in the table below.

| Volume of activated antibody (µL) | Volume of activated oligo (µL) | Volume of Wash Buffer (µL) | Antibody : oligo starting molar ratio |
|---|---|---|---|
| 300 | 300 | 0 | 1:15 |
| 300 | 200 | 100 | 1:10 |
| 300 | 100 | 200 | 1:5 |
| 300 | 60 | 240 | 1:3 |

***Δ Note:*** *The antibody : oligo ratio is an average since a population of labeled antibodies will be produced following the conjugation reaction. Each antibody will not have exactly the same number of oligos bound to it.*

4.5.2 Mix and incubate at room temperature for 1 hour. Conjugations can also be incubated overnight at room temperature with no adverse effect.

4.5.3 Your conjugate is now ready for use. You may also purify the conjugate to remove any unbound oligos if this is required for your application (see section 4.6).

4.5.4 Any unused activated oligo may be stored at - 20°C.

## 4.6 Conjugate Purification

*Δ* ***Note:*** *50 µg of antibody is the lower limit for seeing a clearly visible pellet.*

4.6.1 Warm the Conjugate Clean Up Reagent by placing the tube in warm water (not warmer than 40°C) for 10 minutes and mixing regularly. If the sample does not dissolve completely, spin the sample in a bench top micro-centrifuge at a recommended maximum speed of 13,000 *x g* for 1 minute, and use the supernatant.

4.6.2 Add an equal volume of Conjugate Clean Up Reagent to the volume of antibody/oligo mixture, mix and incubate at room temperature or on ice for 20 minutes. E.g.: add 600 µL of Conjugate Clean Up Reagent to 600 µL of antibody/oligo mixture.

4.6.3 Centrifuge in a bench top micro-centrifuge for 5 minutes at 15,000 *x g*. The required spin time will vary depending on buffer composition and speed. The speed should not exceed 15,000 *x g*. Position the Eppendorf tube in the centrifuge in such a manner that you know where your pellet will be located.

4.6.4 Remove sample from the centrifuge taking care not to dislodge the small pellet at the bottom of the tube. If no pellet is seen add more Conjugate Clean Up Reagent (another 1/10 volume), mix well and incubate on ice for a further 10 minutes and centrifuge. If no pellet is seen when using a protein other than an antibody, add half of the volume of Conjugate Clean Up Reagent added at step 4.6.2, mix well and incubate on ice for a further 10 minutes and centrifuge. E.g.: if 600 µL of Conjugate Clean-Up Reagent were added at step 4.6.2, top up with another 300 µL.

4.6.5 Carefully remove the supernatant and store until efficient precipitation has been confirmed.

4.6.6 Add 100µL of the Antibody Suspension Buffer to the pellet and mix gently.

4.6.7 To remove as much free oligo as possible, a second clean-up should be performed on the same conjugate.

4.6.8 The antibody/oligo conjugate is now ready to use.

## 4.7 Use of Control Oligo and Antibody

Each conjugation kit is supplied with both a control oligo (a 30-base oligo with a 5' terminal amine) and a control antibody (rabbit IgG). These reagents are included as positive controls in order to give the option of confirming the conjugation chemistry is working optimally.

### Procedure for Activating the Control Oligo / Antibody:

4.7.1 Add 100 µL of Wash Buffer to both the lyophilized vials of control oligo and control antibody.

4.7.2 Add the 100 µL of control oligo to a vial of Oligo Activation Reagent. Mix and incubate at room temperature for 30 minutes.

4.7.3 Add the 100 µL of control antibody to a vial of Antibody Activation Reagent. Mix and incubate at room temperature for 30 minutes.

4.7.4 Meanwhile proceed to the desalting procedure (step 4.3).

## 4.8 Conjugate Storage:

The long-term stability of the antibody-oligo conjugate will depend on many factors, including the antibody and oligo themselves, the storage temperature and storage conditions.

In order to maximize stability, we would recommend storing the conjugate in a form that is as concentrated as possible and at a low temperature. We would suggest checking with the antibody and oligo manufacturers if their products can be

stored in 50% glycerol at - 20°C – you should be able to store most conjugates in these conditions, as long as the they are compatible with the unconjugated antibody and oligo. If it is appropriate for your reagents and subsequent experiments, the addition of preservatives may also be helpful.

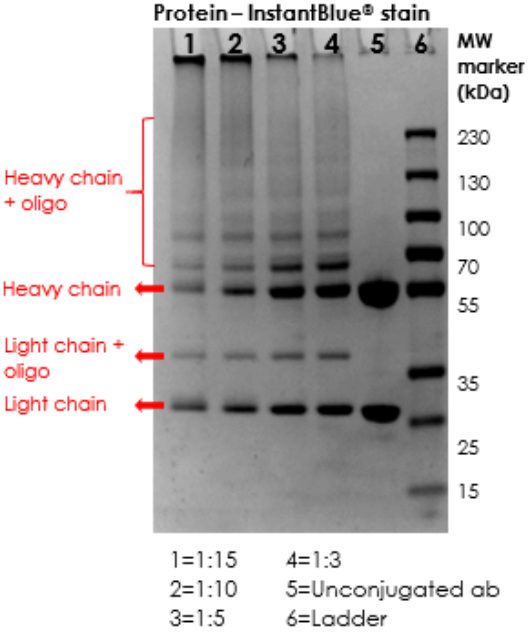
## 5. Analysis of the Antibody-Oligo Conjugate

The antibody-oligo conjugates can be analysed in a number of ways. The best method to confirm conjugation is a positive result in the experiment that the conjugated will be used in. Alternatively, the conjugates can be analysed using gel electrophoresis.

5.1 A small amount (5 -10 µg) of the conjugate can be run on a reducing SDS-PAGE gel. Mix the conjugate sample with 2X gel loading buffer (not supplied) and heat at 100°C for 5 minutes.

5.2 Cool the sample, then load onto a reducing SDS-PAGE gel. A 4-12% gradient gel is recommended for best results. Run the gel under reducing conditions and stain for protein using Coomassie Blue stain or a suitable equivalent. After destaining, the gel can be analyzed for the presence of antibody-oligo conjugates. A typical gel image for IgG is shown below (5 µg of conjugate was used per well).

**Reducing SDS-PAGE after Oligo Conjugation**




***Δ Note:*** *IgG consists of two heavy and two light chains. Not all these chains will be attached to an oligo. There will be a number of unlabelled heavy and light antibody chains even within an excellent conjugate. This is especially true for low ratio conjugates. It is also common to see cross-linking with high ratio conjugates which will impede the migration of the conjugate through the gel.*

***Δ Note:*** *Antibody chains attached to oligos may not stain as efficiently as unlabelled antibody chains. The gel images should therefore be considered as qualitative rather than quantitative.*

***Δ Note:*** *The size of the shift in the heavy chain will depend on the size of the oligo to which it has been conjugated. Larger oligos will generate a larger band shift and vice versa. The oligo type used in the example above is a 30mer.*

***Δ Note:*** *Other antibody subtypes, such as IgM, will generate a different banding pattern on the gel.*

# Notes

# Technical Support



**For all technical or commercial enquiries please go to:**

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