Innova Biosciences
making science easier

Thunder-Link® oligo conjugation system

Applicable to:

420-0000 1 reaction (to conjugate 100µg antibody)

420-0300 3 reaction (to conjugate 100µg antibody each)

Release 8 05/01/2015

Introduction

Antibody-oligonucleotide conjugates are the next generation of tools in biomarker detection, overcoming sensitivity and linear range issues often encountered with standard antibody labels. Antibody-oligonucleotide conjugates also have the potential to be the platform tool in multiplexed protein diagnostic assays. The Thunder-Link® oligo conjugation system allows antibody oligo conjugates to be generated very easily and efficiently, while the inclusion of positive controls enables the end user to confirm that the conjugation chemistry is working correctly.

Shipping and storage

This kit is shipped at ambient temperature. Upon receipt, store the Activation Reagents and buffers pack at 2-8°C and the Clean Up Reagent and columns at room temperature.

Kit contents

2 or 4 glass vials of Thunder-Link Oligo Activation Reagent (depending on pack size)

2 or 4 glass vials of Thunder-Link Antibody Activation Reagent (depending on pack size)

1 vial of freeze dried control oligo

1 vial of freeze dried control antibody

4 or 8 separating columns

1 x Thunder-Link Wash Buffer (80ml or 160ml)

1 or 2 vials of Thunder-Link Conjugate Clean Up Reagent*

1 or 2 vials of Thunder-Link Antibody Suspension Buffer

*This buffer may form crystals on storage. In the event of this happening simply warm the buffer to 40°C and shake vigorously to re-dissolve contents.

Once dissolved, maintain the tube at ~22°C to prevent further crystal formation before use.

Buffer considerations

Buffer components	Oligonucleotide buffer	Antibody buffer
pH	6-8	7-9
Amine free buffer *(ideally phosphate buffer)*	✓	✓
Non-buffering salts *(e.g. sodium chloride)*	✓	✓
Chelating agents *(e.g.EDTA)*	✓	✓
Sugars	✓	✓
Glycerol	<50%	<50%
Thiomersal	×	×
Thimerosal	×	×
Merthiolate	×	×
Sodium Azide*	<0.1%	<0.1%
BSA*	<0.1%	<0.1%
Gelatin*	<0.1%	<0.1%
Tris	<20mM	<20mM
Glycine	×	×
Nucleophilic components *(Primary amines e.g. amino acids or ethanolamine and thiols e.g. mercaptoethanol or DTT)*	×	×

[1] ***Please note that individually the concentrations shown should not affect the reaction. However in combination with additional compounds that are not recommended above a certain concentration, the reaction may be affected.***

Instructions

1. Activation of Oligo

Recommended concentration and size of oligo for optimal results:

The oligo must be between 20 and 120 base pairs in length 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.

The oligo must be HPLC purified and be 60-100µM concentration and be in 100µl of a suitable buffer. If the oligo concentration is greater than 100µM, dilute to 100µM in Wash Buffer. For conditions outside these recommendations, contact our Technical Support Team for advice.

Oligo Activation Procedure

Add 100µl of the oligo into the Oligo Activation Reagent vial. Mix gently and incubate for 1 hour at room temperature.

Meanwhile proceed to desalting procedure (step 3).

2. Activation of Antibody

Recommended antibody concentration and quantity of antibody for optimal results:

The antibody to be activated must be purified and at a concentration of 1mg/ml. Higher antibody concentrations should be diluted to 1mg/ml with wash buffer.

With antibody concentrations below 1mg/ml the antibody must be concentrated before use.

The kits are designed to activate 100µg of antibody in 100µl of a suitable buffer. For conditions outside these recommendations, contact our Technical Support Team for advice.

Antibody activation procedure

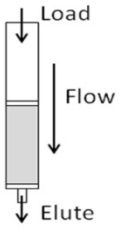
Add 100µl of the 1mg/ml antibody into the Antibody Activation Reagent vial. Mix gently and incubate for 1 hour at room temperature.

Meanwhile proceed to desalting procedure (step 3).

3. Desalting Procedure

Use one column per Desalting. The columns are designed for single use. Discard after use.

1) Secure a separating column in a vertical position. Remove the two caps and allow the storage liquid to flow through the column to waste (remove the upper cap first).



2) Equilibrate column by adding 3ml of Wash Buffer to the top of the column and allow the liquid to flow through under gravity. Discard the flow-through. Repeat a further 4 times.

3) After the 1 hour incubation, add the 100µl of activated material (oligo or antibody) to the top of the column and allow the liquid to completely absorb into the column.

4) Add 600µl of Wash Buffer to the top of the column. This liquid is required to push the activated material to the base of the column. Allow this liquid to completely absorb before proceeding to the next step.

5) Place a collection vessel (not supplied) under the column. Add 200µl of Wash Buffer to the top of the column.

6) Collect the eluate from the column into the clean tube. This column eluate contains the activated material which is now free of Activation Reagent and ready to use.

Storage of activated material

Oligo and control oligo

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

Antibody and control antibody

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

Generation of Purified Antibody / Oligo Conjugate

This kit can be used to generate antibody:oligo conjugates with a range of different antibody:oligo ratios. This can be achieved simply by adding different amounts of oligo to the antibody as shown in the table below. The preferred ratio will depend upon your application, and may need to be determined experimentally (see optimisation instructions on page 3).

Conjugation:

1) Add the 200µl 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 ratio*
200	200	0	1:15
200	133	67	1:10
200	67	133	1:5
200	40	160	1:3
200	13	187	1:1

The ratio of oligonucleotide to antibody is only ever an average for the population of labeled molecules, as individual molecules may have different amount of oligonucleotide incorporated into them.

2) Mix and incubate at room temperature overnight.

3) Your conjugate is now ready for use. You may also purify the conjugate to remove any unbound oligo if this is required for your application (see conjugate purification information on page 3).

4) Any unused activated oligo may be stored frozen.

Optimization of the antibody:oligo ratio

If you need to test which antibody:oligo ratio is best for your application this can be achieved by reducing the volume of antibody used and running several experiments using different ratios.

1) Mix together the volumes of antibody, 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 ratio*
50	50	0	1:15
50	33	17	1:10
50	17	33	1:5
50	3	47	1:1

The ratio of oligonucleotide to antibody is only ever an average for the population of labeled molecules, as individual molecules may have different amount of oligonucleotide incorporated into them.

2) Incubate overnight at room temperature

3) Your conjugates are now ready for use. You may also purify the conjugate to remove any unbound oligo if this is required for your application – see conjugate purification information on page 3).

4) Any unused activated oligo may be stored frozen.

Conjugate purification

Purification of 400µl of conjugate:

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. Make sure any crystals have been re-dissolved, shake if necessary.
2) Add 320µl of Conjugate Clean Up Reagent to the antibody/oligo mixture, mix and incubate at room temperature or on ice for 10 minutes.
3) Centrifuge in a bench top microcentrifuge for 5 minutes at 15,000g.
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.
5) Carefully remove the supernatant and store until efficient precipitation has been confirmed.
6) Add 100µl of the Antibody Suspension Buffer to the pellet and mix gently.
7) The antibody / oligo conjugate is now ready to use.

Purification of 100µl of conjugate:

Caution: As the amount of material being handled is very small this purification procedure is much more technically demanding.

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. Make sure any crystals have been re-dissolved, shake if necessary.
2) Add 80ul of Conjugate Clean Up Reagent to the 100µl of antibody/oligo mixture, mix and incubate at room temperature or on ice for 10 minutes.
3) Centrifuge in a bench top microcentrifuge for 5 minutes at 15,000g. Position the Eppendorf tube in the centrifuge in such a manner that you know where any pellet will be located.
4) Remove sample from the centrifuge taking care not to dislodge the very 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.
5) Carefully remove the supernatant and store until efficient precipitation has been confirmed.
6) Add 50µl of the Antibody Suspension Buffer to the pellet and mix gently.
7) The antibody / oligo conjugate is now ready to use.

Use of the Control Oligo and Antibody (optional)

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

1) Add 100µl of Wash Buffer to both the lyophilized vials of control oligo and control antibody.
2) Add the 100µl of Control Oligo to a vial of Oligo Activation Reagent. Mix and incubate at room temperature for 1 hour.
3) Add the 100µl of control antibody to a vial of Antibody Activation Reagent. Mix and incubate at room temperature for 1 hour.
4) Meanwhile proceed to the desalting procedure (step 3).

Note you will require two columns. One column is required for the control oligo and a second column is for the control antibody.

8.2 Procedure for generating a Control Antibody / Oligo conjugate

The activated Control Oligo is conjugated to the activated Control Antibody using the procedure described in the protocol.

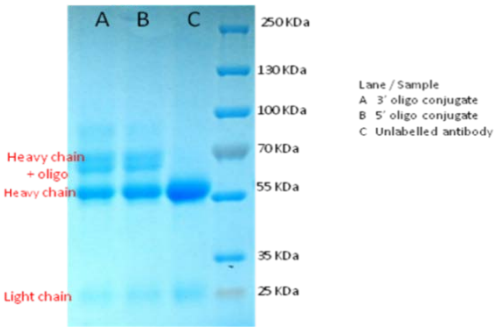
Analysis of the Antibody / Oligo conjugate

The generated conjugates can be analyzed in a variety of ways. The best method to confirm conjugation is a positive result in the chosen application. Alternatively, the conjugates can be analyzed using Gel Electrophoresis.

A small amount (5 to 10µg) of the conjugate can be run on a reducing SDS/PAGE gel. The small sample of conjugate should be mixed with the 2X gel loading buffer (not supplied) and heated at 100°C for 5 minutes. This treatment will break all the disulphide bonds present in the antibody.

The sample should be allowed to cool before being loaded onto the SDS/PAGE gel (not supplied). A 4 to 12% gradient gel is recommended for best results. The gel is then run and stained for protein using coomassie blue stain or a suitable equivalent. After destaining the gel can be imaged to reveal the presence of antibody / oligo conjugates. A typical gel image for an IgG is shown below.

Reducing SDS-PAGE after Oligo Conjugation



Note IgG consists of two heavy and two light chains. Not all of these chains will be attached to an oligo. There will be a number of unlabeled heavy and light antibody chains even within an excellent conjugate. This is especially true for low ratio conjugates.

Antibody chains attached to oligos may not stain as efficiently as unlabeled antibody chains. The gel images should therefore be considered as qualitative rather than quantitative.

The size of the shift in the heavy chain will depend on the size of the oligo conjugated. Larger oligos will generate a larger band shift and smaller oligos a smaller shift. The oligo used in the example is a 30mer. Other antibody subtypes, such as IgM will generate a different banding pattern on the gel.

Frequently asked questions

Q1. Can I use the kit to conjugate oligos to proteins other than antibodies?

The Thunder-Link® oligo conjugation system is primarily designed to conjugate oligos to purified antibodies. However, because the system works by targeting 'available' amine groups on the antibody it can be used to label other proteins and peptides. Please contact our technical support team for specific advice.

Q2. Does antibody species or subtype make a difference to the conjugation efficiency?

No. The Thunder-Link® oligo conjugation system is primarily designed to conjugate oligos to purified IgG. The kit will conjugate oligos to IgG irrespective of species. The kit will also conjugate all other antibody sub types. Please contact our technical support team for specific advice.

Q3. Do I need a specific functional groups on my Oligo?

Yes. The oligo MUST contain a terminal amine group. This amine group is added during oligo synthesis and may be either 5' or 3'. Generally 5' oligos conjugate slightly more efficiently than 3' oligos, although the difference is minimal.

Q4. Is there a limit to the size of my oligo?

Yes. The kit is designed to conjugate oligos between 20 and 120 bases. Optimal results are obtained with oligos of around 40 bases. For oligos outside of this size range please contact our technical support team for advice.

Q5. Does my oligo have to be HPLC purified?

Yes. The oligo MUST be HPLC purified as some of the chemicals used in oligo synthesis can interfere and inhibit the conjugation chemistry.

Q6. Can I use the Thunder-Link® to conjugate double stranded DNA to antibodies?

Yes however you must ensure only one end of the DNA strand is amine modified.

Q7. Are larger pack sizes available?

Yes. The standard kit is designed to conjugate 100µg of antibody. Innova Biosciences can provide larger kits on request. Please contact our customer service team at info@innovabiosciences.com

Q8. Does Innova Biosciences offer a custom oligo conjugation service?

Yes. Innova Biosciences does offer a custom oligo conjugation service. Our custom conjugation laboratory offers a wide variety of services including oligo conjugations. Please see our web site for full details of available services or contact us at info@innovabiosciences.com

Technical Support

Tel: +44(0)1223 496175

E-mail: technical.enquiries@innovabiosciences.com