Antibody-Oligonucleotide All-In-One™ Conjugation Kit

Cat. No.: A-9202-001

Storage: 2° – 8°C — Do Not Freeze.

The Antibody-Oligonucleotide All-In-One Conjugation Kit requires 100 µg antibody at a concentration of 1 mg/mL. The antibody buffer should be free of carrier proteins such as BSA or gelatin and should not contain a high concentration (>25%) of glycerol. The kit is designed to perform optimally with 25 OD_{260} units of amino-modified oligo in the 20 – 60 nucleotide range. Oligos shorter than 20 nucleotides cannot be successfully conjugated with this kit; oligos longer than 60 nucleotides may be used, albeit at the expense of conjugate yield. A minimum of 15 OD_{260} units of amino-modified oligo may be used if required.

DESCRIPTION

The Antibody-Oligonucleotide All-In-One Conjugation Kit contains all necessary reagents and components to produce one antibody-oligonucleotide conjugate. Based on patented **SoluLINK® bioconjugation technology**, it allows any purified mammalian antibody of the IgG isotype to be conjugated and purified in a three-stage process taking around 11 hours and involving just 2 hours of hands-on time. First, the user-supplied amino-oligo is modified with S-4FB, then the user-supplied antibody is modified with S-HyNic. Next, the two modified biomolecules are mixed in the presence of a reaction catalyst to form the conjugate, which is subsequently purified using a magnetic affinity solid phase. This procedure results in 20 – 60 µg of high purity antibody-oligonucleotide conjugate that is ready for use (Figure 1). Yield is largely dependent on oligonucleotide length, with shorter oligonucleotides typically having higher yields. Final conjugate concentrations typically range from 0.1 – 0.3 mg/mL.

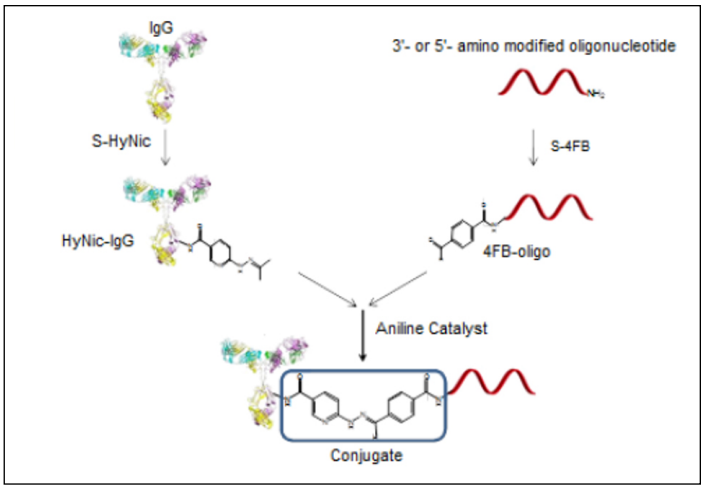

Figure 1. Reaction of a HyNic-modified IgG with a 4FB-modified oligonucleotide leads to the rapid formation of a stable antibody-oligonucleotide conjugate.

KIT COMPONENTS

Component	Amount
S-HyNic	100 µg
Oligo Resuspension Solution	1 mL
1X Modification Buffer	1.5 mL
Bead Wash Buffer A	5 mL
Bead Elution Buffer A	250 µL
Red Cap Spin Column	2
Yellow Cap Spin Column A	1
Blue Cap Spin Columns D	2
Brown Cap Spin Column C	2
Anhydrous DMF	1.5 mL
2-Hydrazinopyridine (2-HP) Reagent	500 µL
Affinity Magnetic Beads	100 µL
2 mL Collection Tubes	14
S-4FB	1 mg

PROTOCOL

The Antibody-Oligonucleotide All-In-One Conjugation Kit follows a three-stage protocol, where each stage takes several hours to complete. If desired, the protocol can be split over two days, with stage 1 (modification of the amino-oligo with S-4FB, 4-hour duration) being performed on day 1, and stages 2 and 3 (modification of the antibody with S-HyNic followed by conjugate formation and purification, 6.5-hour duration) on day 2. It is not recommended to stop the procedure after stage 2. Starting with a 4FB-labeled oligo greatly reduces the overall time to complete the process.

Stage 1: Modification of amino-oligo with S-4FB

If starting with a 4FB-modified oligo, proceed directly to Stage 2.

A. Enter amino-oligo details into the Antibody-Oligonucleotide Conjugation Calculator

Enter the following parameters directly from the oligo vendor's Certificate of Analysis into section A of the Antibody-Oligonucleotide Conjugation Calculator.

a) Oligo name

b) OD_{260} units supplied by vendor

c) Oligo molar extinction coefficient (liter mol^{-1} cm^{-1})

d) Oligo molecular weight (Daltons)

e) Nanomoles per OD_{260} as listed on the product data sheet

B. Resuspend amino-oligo

1. Ensure at least 15 OD_{260} units of oligo are available for modification.
2. Centrifuge the vial containing lyophilized oligo at 15,000 x g for 15 seconds to pellet the lyophilizate at the bottom of the tube.

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3. If the tube contains between 15 and 25 OD_{260} units of oligo, add 50 µL of Oligo Resuspension Solution to the tube. If the tube contains more than 25 OD_{260} units of oligo, add a sufficient volume of Oligo Resuspension Solution to create a 0.5 OD_{260}/µL solution (e.g., if there are 31 OD_{260} units of oligo, add 62 µL of Oligo Resuspension Solution).
4. Allow the pellet to rehydrate for 1 minute, then gently vortex the solution on medium speed for 10 seconds to assist dissolution. Repeat this process until no undissolved lyophilizate remains.

C. Measure oligo concentration

The oligo concentration can be measured using either a micro-volume UV-Vis spectrophotometer (e.g., NanoDrop™) or a conventional UV-Vis spectrophotometer. Follow the instructions below for the type of instrument available.

Determining oligo concentration with a NanoDrop (models ND-1000 and ND-2000):

1. In a microcentrifuge tube, prepare a 1:200 dilution by transferring 2.0 µL of oligo solution into 398 µL of ultrapure water. Mix well by vortexing.
2. Select the "Nucleic Acid" module on the NanoDrop and initialize the instrument using ultrapure water (NanoDrop 1000 only).
3. Clean the sample pedestal and blank the instrument with 2 µL of ultrapure water.
4. Measure the 260 nm absorbance of the 1:200 oligo solution as displayed in the 10-mm pathlength window.
5. Divide the A_{260} value by 5 to calculate the OD_{260}/µL concentration of the stock oligo solution.

Determining oligo concentration with a conventional UV-Vis spectrophotometer:

1. In a microcentrifuge tube, prepare a 1:500 dilution by transferring 2.0 µL of oligo solution into 998 µL of ultrapure water. Mix well by vortexing.
2. Using a 1 cm pathlength quartz cuvette, blank the spectrophotometer at 260 nm using ultrapure water.
3. Measure the 260 nm absorbance of the 1:500 oligo solution.
4. Divide this number by 2 to calculate the OD_{260}/µL concentration of the stock oligo solution.

After the OD_{260}/µL amino-oligo concentration is calculated by either method above, multiply that value by 50 µL to determine the amount of oligo available for modification. If less than 15 OD_{260} units are available, obtain additional oligo before proceeding.

D. Buffer exchange amino-oligo

1. Prepare a red cap spin column by loosening the cap one-half turn, twisting off the bottom closure, and placing it in an empty 2 mL collection tube.
2. Using a lab marker, place a vertical line on the outside of the column. Ensure that this line faces outward (away from the center of the rotor) in this and all subsequent steps.
3. Centrifuge the column at 1,500 x g for 1 minute to remove storage buffer.

   Important: *Ensure the centrifuge is set to "g" or RCF rather than RPM in all centrifugation steps.*
4. Remove the column from the collection tube (discard the collection tube containing excess buffer) and place the column in a new 2 mL collection tube.
5. Slowly and carefully pipet exactly 50 µL of oligo solution into the center of the resin bed. Be careful not to let the oligo solution contact the tube wall; it must channel down through the resin itself.
6. Replace the cap and loosen it one-half turn.
7. Centrifuge the column at 1,500 x g for 2 minutes to recover the desalted oligo into the collection tube.
8. Transfer this solution into a new microcentrifuge tube and measure the volume with a P-100 pipet.
9. Enter the volume (µL) of desalted oligo recovered into section B of the Antibody-Oligonucleotide Conjugation Calculator.
10. Gently vortex the oligo solution to mix thoroughly.
11. Repeat the concentration measurement as described in section C above.
12. Enter the calculated OD_{260}/µL stock oligo concentration into section B of the Antibody-Oligonucleotide Conjugation Calculator.

    Note: *Excess undesalted oligo may be stored indefinitely at or below -20°C.*

E. Dissolve S-4FB

1. Briefly centrifuge the S-4FB reagent at 15,000 x g to ensure that all material is at the bottom of the tube.
2. Add 40 µL of Anhydrous DMF to the S-4FB and vortex for 20 seconds to resuspend.
3. Continue to periodically vortex until the pellet is completely dissolved. It may be necessary to pipet the sample up and down several times.
4. Briefly spin the completely dissolved reagent to the bottom of the tube.

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F. Modify amino-oligo with S-4FB

Using the information entered in sections A and B, the Antibody-Oligonucleotide Conjugation Calculator will determine the volumes of Anhydrous DMF and S-4FB in Anhydrous DMF to be added to the desalted amino-oligo solution. These volumes can be found in section C of the Antibody-Oligonucleotide Conjugation Calculator.

1. Add the indicated volume (µL) of Anhydrous DMF to the oligo solution and briefly vortex to mix.
2. Add the indicated volume of S-4FB dissolved in Anhydrous DMF to the oligo and vortex to mix. Do not centrifuge the reaction mixture after the S-4FB reagent has been added.
3. Incubate at room temperature for 2 hours to allow the S-4FB to react with the amino-oligo.
4. Centrifuge the tube at 15,000 x g for 2 minutes to pellet any insoluble reaction by-products. In Part G below, use only the clear supernatant (which contains the 4FB-oligo) in the desalting reactions.

G. Remove excess S-4FB

1. Five minutes prior to the end of the 4FB-oligo modification reaction, prepare two brown cap spin columns by loosening each cap one-half turn, twisting off each bottom closure, and placing each column in an empty 2 mL collection tube.
2. Using a lab marker, place a vertical line on the outside of each column. Ensure that this line faces outward (away from the center of the rotor) in this and all subsequent steps.
3. Centrifuge the columns at 1,500 x g for 1 minute to remove storage buffer.

   Important: *Ensure the centrifuge is set to "g" or RCF rather than RPM in all centrifugation steps.*
4. Remove the columns from the collection tubes (discard the collection tubes containing excess buffer) and place the columns in new 2 mL collection tubes.
5. Slowly and carefully pipet the entire oligo modification reaction into the center of only one of the columns. Be careful not to let the oligo solution contact the column wall; it must channel down through the resin itself.
6. Replace the cap and loosen one-half turn. Leave the other column on the bench top during the next step (do not use it as a balance tube).
7. Balance the centrifuge with a microcentrifuge tube containing water and spin the column containing modified oligo at 1,500 x g for 2 minutes.
8. Immediately transfer the entire eluate from the step above to the other brown cap spin column and repeat the desalting process by centrifuging at 1,500 x g for 2 minutes. This "double-desalting" will ensure that all traces of un-incorporated 4FB are removed from the oligo.
9. Transfer the desalted oligo solution to a 1.5 mL microcentrifuge tube while measuring the volume with a P-200 pipet.
10. Vortex the solution to mix thoroughly before proceeding to Part H.

H. Measure 4FB-oligo concentration

Measure the 4FB-oligo concentration using the procedure described in part C above. Enter the 4FB-oligo concentration (OD_{260}/µL) in section D of the Antibody-Oligonucleotide Conjugation Calculator.

I. Quantify 4FB Molar Substitution Ratio (MSR) - optional

The 4FB Molar Substitution Ratio assay quantifies the amount of 4FB attached to the oligo. It is performed by reacting an aliquot of 4FB-oligo with 2-Hydrazinopyridine (2-HP) reagent at 37°C for 60 minutes, after which the A_{360} of the sample is measured. This assay ensures that the oligo is both 4FB-modified and properly buffer exchanged. It can be performed using either a micro-volume UV-Vis spectrophotometer (e.g., NanoDrop) or a conventional UV-Vis spectrophotometer. Follow the instructions below for the type of instrument available.

<u>NanoDrop 4FB Molar Substitution Ratio assay:</u>

1. Prepare a 2-HP blank solution by adding exactly 2.0 µL of water to 18.0 µL of 2-HP reagent in a microcentrifuge tube. Label this tube "2-HP Blank."
2. Prepare a 4FB-oligo MSR sample by adding exactly 2.0 µL of 4FB-modified oligo to 18.0 µL of 2-HP reagent in a microcentrifuge tube. Label this tube "4FB-Oligo MSR."
3. Vortex both solutions to mix, then briefly spin the tubes to pool the contents to the bottom of each tube.
4. Incubate both tubes at 37°C for 60 minutes.
5. After the incubation period, centrifuge both tubes at 15,000 x g for 15 seconds to collect condensation at the bottom of the tubes, then vortex to mix.
6. Launch the NanoDrop UV-Vis module. Initialize the instrument with 2 µL of ultrapure water (NanoDrop ND-1000 only).
7. Blank the NanoDrop with 2 µL of 2-HP Blank and clean the pedestal.
8. Set the λ1 absorbance wavelength to 360 nm.
9. Place a 2 µL aliquot of the 4FB-Oligo MSR reaction on the pedestal and click the "Measure" icon. The 1-mm A360 absorbance will appear.
10. Enter this value directly into the Antibody-Oligonucleotide Conjugation Calculator (Section E: 4FB-Oligo MSR Assay).

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Conventional UV-Vis spectrophotometer 4FB Molar Substitution Ratio assay (≤ 200 µL micro-cuvette):

1. Prepare a 2-HP Blank and 4FB-Oligo MSR sample by following steps 1 through 5 in the NanoDrop method above.
2. Prepare a 1:10 dilution of the 2-HP Blank and 4FB-Oligo MSR reactions by adding 180 µL of ultrapure water to each tube. Vortex to mix.
3. In a 1-cm pathlength quartz micro-cuvette, blank the spectrophotometer at 360 nm with the diluted 2-HP Blank.
4. Measure the 360 nm absorbance of the diluted 4FB-Oligo MSR sample.
5. Enter this value directly into the Antibody-Oligonucleotide Conjugation Calculator (Section E: 4FB-Oligo MSR Assay).

Conventional UV-Vis spectrophotometer 4FB Molar Substitution Ratio assay (1 mL cuvette):

1. Prepare a 2-HP Blank and 4FB-Oligo MSR sample by following steps 1 through 5 in the NanoDrop method above, except adding 10 µL of water for the Blank and 10 µL of 4FB-Oligo for the MSR reaction into 90 µL each of 2-HP reagent.
2. Prepare a 1:10 dilution of the 2-HP Blank and 4FB-Oligo MSR reactions by adding 900 µL of ultrapure water to each tube. Vortex to mix.
3. In a 1-cm pathlength quartz cuvette, blank the spectrophotometer at 360 nm with the diluted 2-HP Blank.
4. Measure the 360 nm absorbance of the diluted 4FB-Oligo MSR sample.
5. Enter this value directly into the Antibody-Oligonucleotide Conjugation Calculator (Section E: 4FB-Oligo MSR Assay).

An MSR value of ≥ 0.5 4FB groups per oligo is required to proceed with the conjugation reaction.

Stage 2: Modification of antibody with S-HyNic

A. Prepare antibody

100 µg (100 µL) of antibody is required for the conjugation reaction. Use 1X Modification Buffer to dissolve lyophilized antibody or dilute aqueous antibody to a concentration of 1 mg/mL. If aqueous antibody is at less than 1 mg/mL, it must be concentrated using a centrifugal diafiltration apparatus or similar device (not supplied). Refer to the manufacturer's instructions for use.

B. Buffer exchange antibody

1. Prepare a red cap spin column by loosening the cap one-half turn, twisting off the bottom closure, and placing it in an empty 2 mL collection tube.
2. Using a lab marker, place a vertical line on the outside of the column. Ensure that this line faces outward (away from the center of the rotor) in this and all subsequent steps.
3. Centrifuge the column at 1,500 x g for 1 minute to remove storage buffer.

   Important: *Ensure the centrifuge is set to "g" or RCF rather than RPM in all centrifugation steps.*
4. Remove the column from the collection tube (discard the collection tube containing excess buffer) and place the column in a new 2 mL collection tube.
5. Slowly and carefully pipet the antibody solution into the center of the resin bed. Be careful not to let the antibody solution contact the tube wall; it must channel down through the resin itself.
6. Replace the cap and loosen one-half turn.
7. Centrifuge at 1,500 x g for 2 minutes.
8. Transfer the buffer-exchanged antibody solution from the bottom of the collection tube to a new 1.5 mL microcentrifuge tube while measuring the volume (µL) recovered.
9. Enter the volume of antibody solution recovered into Section F of the Antibody-Oligonucleotide Conjugation Calculator.
10. Using either a micro-volume UV-Vis spectrophotometer (e.g., NanoDrop) or a conventional UV-Vis spectrophotometer with a 100 µL micro-cuvette, measure the 1-cm pathlength 280 nm absorbance value of the antibody solution.

    Important: *If using a 100 µL micro-cuvette, be sure to recover the antibody sample from the cuvette.*
11. Enter the A_{280} value into Section F of the Antibody-Oligonucleotide Conjugation Calculator.

    Note: *A concentration of 1.0 ± 0.2 mg/mL is required to proceed. If the concentration is outside of this range either obtain additional antibody or adjust the concentration to 1.0 mg/mL with 1X Modification Buffer.*
12. Ensure the following information is entered in section F of the Antibody-Oligonucleotide Conjugation Calculator:

    a) Antibody name

    b) A_{280} of the antibody solution

    c) Volume of antibody solution (µL)

C. Dissolve S-HyNic

1. Briefly centrifuge the 100 µg vial of S-HyNic at 10,000 x g to pellet the linker at the bottom of the tube.
2. Add 35 µL of Anhydrous DMF to the vial of S-HyNic reagent. Pipet the solution up and down for 1 minute to dissolve the pellet. Ensure there are no particulates remaining.

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D. Modify antibody with S-HyNic and buffer exchange

1. Add 2.0 µL of dissolved S-HyNic linker to the antibody sample. Immediately pipet the solution up and down, and gently vortex or flick the vial to mix.
2. Incubate the antibody-HyNic modification reaction at room temperature for 2 hours.
3. Five minutes prior to the end of the HyNic modification reaction, prepare a yellow cap spin column by loosening the cap one-half turn and twisting off the bottom closure.
4. Place the spin column into a 2 mL collection tube.
5. Using a lab marker, place a vertical line on the outside of the column. Ensure this line faces outward (away from the center of the rotor) in this and all subsequent steps.
6. Centrifuge the column at 1,500 x g for 1 minute to remove storage buffer.

   Important: *Ensure the centrifuge is set to "g" or RCF rather than RPM in all centrifugation steps.*
7. Remove the column from the collection tube (discard the collection tube containing excess buffer) and place the column in a new 2 mL collection tube.
8. Slowly and carefully pipet the HyNic-antibody solution into the center of the resin bed. Be careful not to let the HyNic-antibody solution contact the tube wall; it must channel down through the resin itself.
9. Replace the cap and loosen one-half turn.
10. Centrifuge at 1,500 x g for 2 minutes.
11. Transfer the HyNic-modified antibody from the bottom of the collection tube to a new labeled 1.5 mL microcentrifuge tube while measuring the volume (µL) recovered with a P-200 pipet.

Proceed immediately to Stage 3, conjugate formation.

Stage 3: Conjugate formation and purification

A. Form conjugate

1. Enter the name of the antibody and the name of the oligo into section G of the Antibody-Oligonucleotide Conjugation Calculator.

   Note: *Section G uses data from other sections to calculate the volume of 4FB-oligo required. Please ensure the light green fields from other sections are populated with the correct data.*
2. Add the indicated volume (µL) of 4FB-modified oligo displayed in section G of the Antibody-Oligonucleotide Conjugation Calculator to the HyNic-modified antibody. Pipet the solution up and down, then gently flick the tube to mix.
3. Incubate the antibody-oligo conjugation reaction for 2 hours at room temperature.

B. Purify conjugate

1. Centrifuge the vial containing affinity magnetic beads (black slurry) at 1,000 x g for 5 seconds to collect the bead contents at the bottom of the tube.
2. Add 500 µL of Bead Wash Buffer to the bead slurry using a P-1000 pipet and pipet the solution up and down several times to mix.
3. Quickly, before the beads settle, place the tube on a magnet for 10 seconds.
4. Carefully remove and discard the clear supernatant using a P-200 pipet without disturbing the pellet.
5. Repeat steps 2 – 4 three additional times to fully wash the beads, removing the supernatant after each wash.
6. Immediately add the conjugation reaction (approximately 115 µL) directly to the washed bead pellet.
7. Gently pipet the slurry/conjugate suspension up and down 3 to 4 times with a P-1000 pipet set to 100 µL. Keep the same pipet tip on the pipet for the following three steps.
8. Allow the slurry to incubate for 10 minutes away from the magnet.
9. Repeat the resuspension step three additional times for a total conjugate binding time of 40 minutes with four mixing cycles. Some minor but unavoidable bead loss can occur due to nonspecific binding of beads inside the pipet tip.
10. Gently pipet the settled slurry up and down one last time and immediately place the slurry on the magnet for 10 seconds before the beads have a chance to settle.
11. The conjugate is now bound to the affinity matrix. Using a P-200 pipet, carefully remove the clear supernatant to a new microcentrifuge tube without disturbing the magnetized bead pellet. Label this fraction "unconjugated oligo."
12. Add 500 µL of Bead Wash Buffer to the bead pellet, remove the tube from the magnet, and pipet the slurry up and down with a P-1000 several times to wash (do not vortex the beads).
13. Before the beads settle, place them back on the magnet for 10 seconds to pellet.
14. Remove and discard the clear supernatant without disturbing the pellet.
15. Repeat the wash step three additional times using 500 µL of Bead Wash Buffer each time. Discard the wash supernatant between washes.
16. Remove the tube from the magnet and add 100 µL of Bead Elution Buffer directly to the bead pellet.
17. Using a P-1000 pipet set to 100 µL, pipet the slurry up and down until the bead pellet adhered to the wall is rinsed to the bottom of the tube.

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18. Incubate the settled slurry for 5 minutes away from the magnet.
19. Mix the slurry up and down to disperse the beads and incubate for another 5 minutes away from the magnet.
20. Repeat the above step one additional time. The total conjugate elution time for these three elution/incubation periods is 15 minutes.
21. Pipet the settled slurry up and down one last time and immediately place it on the magnet for 10 seconds before the beads have a chance to settle.
22. Without disturbing the pellet, carefully transfer the clarified supernatant (approximately 100 µL) containing the eluted conjugate to a new labeled 1.5 mL microcentrifuge tube.
23. Repeat steps 16 – 22 one additional time, pooling the two 100 µL conjugate fractions together in the same tube (approximately 200 µL final volume).

C. Buffer exchange conjugate into storage buffer

1. Prepare two blue cap spin columns by loosening the caps one-half turn, twisting off the bottom closures, and placing each column in an empty 2 mL collection tube.
2. Using a lab marker, place a vertical line on the outside of each column. Ensure this line faces outward (away from the center of the rotor) in this and all subsequent steps.
3. Centrifuge the column at 1,500 x g for 1 minute to remove storage buffer.

   Important: *Ensure the centrifuge is set to "g" or RCF rather than RPM in all centrifugation steps.*
4. Remove the columns from the collection tubes (discard the collection tubes containing excess buffer) and place the columns in new 2 mL collection tubes.
5. Slowly and carefully pipet half (approximately 100 µL) of the conjugate into the center of each resin bed. Be careful not to let the conjugate solution contact the tube walls; it must channel down through the resin itself.
6. Replace the caps and loosen one-half turn.
7. Centrifuge at 1,500 x g for 2 minutes.
8. Pool the two 100 µL eluates containing the purified antibody-oligo conjugate into a single 1.5 mL microcentrifuge tube.
9. Label the tube appropriately for long-term storage.
10. Measure the protein concentration of the conjugate using a Bradford or BCA protein assay as described in the manufacturer's instructions. Bovine gamma globulin protein standards must be used for accurate conjugate concentration results.

STABILITY

Antibody-Oligonucleotide conjugates produced with this kit are stable for at least 1 year when stored at 2 – 8°C.

APPLICATION NOTES

Performing a Bradford assay

Performing a BCA protein assay

Concentrating dilute antibody solutions

Troubleshooting Guide