

Bioconjugation

This guide will take you through the necessary steps to troubleshoot common problems that you may encounter in your bioconjugation process. Below is a list of products associated with this Troubleshooting Guide.

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Bioconjugation

A-9002-001 — HRP Antibody All-in-One™ Conjugation Kit

Problem	Possible Cause	Recommended Action
Poor conjugation yield	Initial antibody concentration and volume were incorrect or unknown.	Whenever possible verify the original antibody concentration using a Bradford protein assay or Thermo Scientific™ NanoDrop™ Spectrophotometer to confirm concentration.
	Antibody concentration was overestimated.	Concentrate or dilute the antibody sample to be conjugated into the required range (4-5 mg/ml and 25 µl).
		Preservatives can interfere with the accuracy of an A_{280} absorbance reading. Remove all interfering preservatives such as thimerosal or Proclin before performing an A_{280} reading.
Poor HyNic modification	Presence of protein carrier (e.g. BSA or gelatin) or large amount of an amine contaminant (e.g. glycine).	Remove all protein carriers such as BSA or gelatin using affinity chromatography or other methods. Buffer exchange the antibody before starting to remove large amounts of amine contaminants such as 100 mM glycine.
Poor HyNic modification	Improper mixing of HyNic reaction components.	Be sure to properly mix the antibody-HyNic reaction mixture. Use a calibrated P-10 pipette to insure accuracy of small volumes.
	Improper storage of S-HyNic reagent can lead to hydrolysis of this NHS ester.	Keep and store S-HyNic sealed in the aluminum pouch provided that contains desiccant.
Low conjugate and/or antibody recovery	Centrifuge set to RPM instead of RCF (or g), or centrifuge out of calibration.	Ensure centrifuge is set to RCF. Use a calibrated variable-speed centrifuge. Follow recommended spin speed/time. Altered spin speeds can adversely affect protein recovery.



Bioconjugation

A-9202-001 Antibody–Oligonucleotide All-in-One™ Conjugation Kit

Problem	Possible Cause	Recommended Action
Poor or undetectable conjugate yield.	Amino-oligonucleotide may not be sufficiently 4FB-modified.	Verify 4FB MSR to ensure proper conjugation. Concentrate 4FB-oligo into the required range (0.3–0.5 OD ₂₆₀ /μl).
	Quality and/or purity of starting oligo is poor.	If antibody quality or quantity is undetermined, perform suitable test such as SDS-PAGE gel analysis and/or a Bradford protein assay to confirm the purity and quantity of the starting material.
	Low buffer exchange spin column recovery volume.	Use a properly calibrated variable-speed centrifuge and follow recommended spin speed/time. Altered spin speeds will adversely compromise recovery. Ensure centrifuge is set to RCF or g, and not RPM.
	Low yield during affinity purification of conjugate.	Be sure to follow all incubation times and number of steps for binding and elution of conjugate.
Poor HyNic modification of antibody.	Presence of protein carriers such as BSA or gelatin may be contaminating antibody sample.	Remove and purify the antibody sample of all protein carriers such as BSA or gelatin using affinity chromatography or other method before proceeding.
	Concentration of S-HyNic modification reagent.	Be sure to thoroughly dissolve S-HyNic reagent before adding it to the antibody. Use a calibrated pipette to ensure accuracy in small volume additions.
	Presence of non-protein amine contaminants.	Remove all non-protein amine contaminants such as glycine or high concentrations of Tris before modifying the antibody with S-HyNic reagent.
	Improper storage of S-HyNic reagent can lead to hydrolysis of the NHS ester.	Keep and store the S-HyNic reagent sealed in foil pouch with desiccant (as provided) at or below 4°C.
	Initial antibody concentration is low.	Confirm initial antibody concentration prior to S-HyNic modification on the spectrophotometer. If in doubt, perform a Bradford assay. Dissolve the antibody sample carefully in the original product vial.
Low oligo 4FB MSR.	Amine contaminants present in oligo preparation	Be sure to use HPLC-purified oligo rather than “crude” or “desalted” preparations.
		Do not omit the Thermo Scientific™ Zeba™ desalting step prior to 4FB modification.



Bioconjugation

B-1001 — ChromaLINK® Biotin (DMF Soluble)

Problem	Possible Cause	Recommended Action
Protein was not biotin labeled or poorly labeled.	Protein has been contaminated with amine containing compounds.	Desalt the protein more thoroughly with a new Zeba Spin column or VivaSpin diafiltration device antibody sample into the required range (i.e., 1 mg/ml and 100 µl).
	The concentration of the protein was too low.	Increase the concentration of the protein to >2.0 mg/ml.
ChromaLINK Biotin was hydrolyzed.	Wet or poor quality DMF/DMSO hydrolyzed the NHS ester.	Use a good quality anhydrous DMF/DMSO to solubilize ChromaLINK.
Molar substitution readings are out of detectable range.	Protein concentrations are out of recommended range.	Concentrate or dilute protein samples into recommend range.
Precipitation of protein on modification.	Precipitation of biotin modified proteins may occur due to over-modification of available lysines and a drastic change in the isoelectric properties of the modified protein.	After the biotinylation reaction is complete, addition of 1M Tris (pH 9.0) can sometimes be used to resuspend the biotinylated protein by adjusting the pH above the pI of the protein.
	Over-modification of the protein.	Reducing the number of equivalents can sometimes prevent precipitation but it will also reduce the MSR.
		Recheck the concentration of ChromaLINK Biotin working stock used to label the protein.



Bioconjugation

B-1007 — Sulfo ChromaLINK® Biotin (Water Soluble)

Problem	Possible Cause	Recommended Action
Protein was not biotin labeled or poorly labeled.	Protein has been contaminated with amine containing compounds.	Desalt the protein more thoroughly with a new Zeba Spin column or VivaSpin diafiltration device.
	The concentration of the protein was too low.	Increase the concentration of the protein to >2.0 mg/ml.
ChromaLINK Biotin was hydrolyzed.	The reagent was left for more than 1 hour in aqueous solution.	Use the ChromaLINK stock solution immediately after preparation.
Molar substitution readings are out of detectable range.	Protein concentrations are out of recommended range.	Concentrate or dilute protein samples into recommend range.
Precipitation of protein on modification.	Precipitation of biotin modified proteins may occur due to over-modification of available lysines and a drastic change in the isoelectric properties of the modified protein.	After the biotinylation reaction is complete, addition of 1M Tris (pH 9.0) can sometimes be used to resuspend the biotinylated protein by adjusting the pH above the pI of the protein.
	Over-modification of the protein.	Reducing the number of equivalents can sometimes prevent precipitation but it will also reduce the MSR.
		Recheck the concentration of Sulfo ChromaLINK Biotin stock solution used to label the protein.

B-1012-010 — ChromaLINK® Biotin Maleimide

Problem	Possible Cause	Recommended Action
Protein was not biotin labeled or poorly labeled.	Protein was not sufficiently reduced using TCEP.	React the Protein with TCEP at the same time as the reaction with the ChromaLINK Maleimide Biotin.
ChromaLINK Biotin Maleimide was hydrolyzed.	Wet or poor quality DMF/DMSO hydrolyzed the maleimido group.	Do not store the ChromaLINK Biotin Maleimide for more than 1 day in DMF solvent.
Molar substitution readings are out of detectable range.	Protein concentrations are out of recommended range.	Concentrate or dilute protein samples into recommend range.
Precipitation of protein on modification.	Precipitation of biotin modified proteins may occur due to over-modification of available lysines and a drastic change in the isoelectric properties of the modified protein.	After the biotinylation reaction is complete, addition of 1M Tris (pH 9.0) can sometimes be used to resuspend the biotinylated protein by adjusting the pH above the pI of the protein.



Bioconjugation

B-9007-009K — ChromaLINK® One-Shot™ Antibody Biotinylation Kit

Problem	Possible Cause	Recommended Action
Poor biotin modification of the antibody.	Initial protein concentration was incorrect.	Follow the recommended procedures only. Concentrate or dilute the antibody sample into the required range (i.e., 1 mg/ml and 100 µl).
	A large excess of non-protein amine contaminants are present in the antibody preparation (e.g., Tris or glycine buffer).	Before labeling, remove all amine contaminants. Some samples are so overly contaminated that exhaustive dialysis or two desalting steps may be required.
	Presence of protein carrier (e.g., BSA or gelatin) contaminated the sample.	Remove and purify away all protein carriers such as BSA or gelatin by affinity or other chromatographic methods. Re-adjust the initial antibody concentration to 1 mg/ml.
	Presence of preservative or other additive may be interfering with an accurate determination of the starting protein concentration.	Do not attempt to label an antibody containing thimerosal. First, remove the preservative, then re-measure and adjust the antibody concentration to 1 mg/ml and 100 µl.
	Presence of residual sodium azide interferes with the labeling reaction.	Refer to recommended Troubleshooting Guide.
ChromaLINK Biotin was hydrolyzed.	Wet or poor quality DMF/DMSO hydrolyzed the NHS ester	Use a good quality anhydrous DMF/DMSO to solubilize ChromaLINK Biotin.
Complete failure of biotin labeling reaction.	Improper mixing of reaction components.	Make sure to properly mix the antibody-ChromaLINK reaction mixture.
	Spectrophotometer lamp output may be low.	Use the biotinylated IgG positive control provided to validate that the spectrophotometer.
	Presences of amine contaminants.	Remove all amine contaminants such as glycine before labeling.
	Improper storage of ChromaLINK Biotin reagent may have caused it to hydrolyze.	Keep ChromaLINK Biotin dessicated at room temperature.
		Follow all recommended procedures.



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Bioconjugation

B-9007-009K — ChromaLINK® One-Shot™ Antibody Biotinylation Kit (continued)

Problem	Possible Cause	Recommended Action
Molar substitution ratio was out of recommended range (3-8 biotins/antibody).	Initial antibody concentration used was too low or too high.	Make sure to properly estimate the initial antibody concentration. Concentrate or dilute the antibody sample into the recommended range, (1 mg/ml in 100 µl), before proceeding.
	Antibody may have precipitated due to over-modification of available lysine residues.	Insufficient mass of antibody.
Low antibody recovery and/or sample precipitation.	Antibody may have aggregated/ precipitated during labeling.	Make sure to add 1 M Tris quench buffer to the labeled sample before final desalting.
	Incorrect antibody concentration antibody was over-modified.	Follow the recommended guidelines.
	Zeba column recovery problem.	Use a calibrated variable-speed centrifuge and spin at recommended speeds and times.



Bioconjugation

B-9007-105K — ChromaLINK® Biotin Protein Labeling Kit

Problem	Possible Cause	Recommended Action
Poor biotin modification of protein.	Initial protein concentration is outside the optimal range for this protocol.	Recheck initial protein concentration. Concentrate or dilute the protein into the required range (e.g., 0.25–10 mg/ml).
	Low number of lysines or sterically hindered lysine residues.	Check the primary sequence of the protein being modified using NCBI Blast to ensure lysine groups are available for modification.
	A large excess of amine contaminants may be present in the protein sample, e.g., Tris or glycine buffers could be contaminating the protein sample.	Buffer exchange the protein sample. Some samples are so overly contaminated that two buffer exchanges may be required before proceeding.
Complete failure of biotin labeling reaction.	Improper mixing of reaction components.	Use the control IgG (unbiotinylated) sample provided and biotinylate according to the recommended protocol
	Improper functioning of spectrophotometer.	UV lamp output low. Recalibrate spectrophotometer.
ChromaLINK Biotin was hydrolyzed	Wet or poor quality DMF/DMSO hydrolyzed the NHS ester.	Use a good quality anhydrous DMF/DMSO to solubilize ChromaLINK Biotin.
Molar substitution ratio are out of detectable range	Protein concentrations are out of recommended range.	Concentrate or dilute protein samples into recommend range.
	Precipitation of biotin-modified proteins may occur due to over-modification of available lysines and a drastic change in the isoelectric properties of the modified protein.	After the biotinylation reaction is complete, addition of 1 M Tris (pH 9.0) can sometimes be used to re-suspend the biotinylated protein. Reduce number of equivalents used to label protein.
Precipitation of protein	Over-modification of the protein.	Check the concentration of ChromaLINK Biotin working stock used to label the protein.
	Critical lysine residues necessary for solubility have been modified.	Attempt to label for shorter periods of time.



Bioconjugation

B-9014-009K — ChromaLINK® Digoxigenin One-Shot™ Antibody Labeling Kit

Problem	Possible Cause	Recommended Action
Poor digoxigenin modification of the antibody.	Initial protein concentration was incorrect.	Follow the recommended procedures only. Concentrate or dilute the antibody sample into the required range (i.e. 1 mg/ml and 100 µl).
	A large excess of non-protein amine contaminants are present in the antibody preparation (e.g. Tris or glycine buffer).	Before labeling remove all amine contaminants. Some samples are so overly contaminated where dialysis or two desalting steps may be required.
	Presence protein carrier (e.g. BSA or gelatin) contaminated the sample.	Remove and purify away all protein carriers such as BSA or gelatin by affinity or other chromatographic methods, re-adjust the initial antibody.
	Presence of preservative or other additive may be interfering with an accurate determination of the starting protein concentration.	Do not attempt to label an antibody containing thimerosal. First remove the preservative then re-measure and adjust the antibody concentration to 1 mg/ml and 100 µl.
	Presence of residual sodium azide interferes with the labeling reaction	Refer to recommended actions in the Troubleshooting Guide.
Complete failure of digoxigenin labeling reaction.	Improper mixing of reaction components.	Make sure to mix the antibody ChromaLINK Digoxigenin reaction mixture completely.
	Improper operation of the spectrophotometer.	Use the digoxigenin-labeled IgG positive control provided to validate that the spectrophotometer is operating properly.
	Presences of amine contaminants.	Remove all amine contaminants such as glycine before labeling.
	Improper storage of the ChromaLINK Digoxigenin reagent may have caused it to hydrolyze.	Store and keep ChromaLINK Digoxigenin in dessicated pouch at room temperature at all times.
Molar substitution ration was out of recommended range (2-8 digoxigenin/antibody).	Initial antibody concentration used was too low or too high.	Make sure to properly determine the initial antibody concentration. Concentrate or dilute the antibody sample into the recommend range (1 mg/ml in 100 µl) before proceeding.
	Antibody precipitated due to over-modification of available lysine residues.	Follow the recommended protocol to insure that low amounts of antibody were not used.



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Bioconjugation

B-9014-009K — ChromaLINK® Digoxigenin One-Shot™ Antibody Labeling Kit (continued)

Problem	Possible Cause	Recommended Action
Low antibody recovery and/or sample precipitation.	Antibody may have been aggregated before it was labeled.	Do not use aggregated samples.
	Incorrect antibody concentration. Antibody was over-modified.	Follow the recommended protocol.
	On rare occasions some antibodies precipitate on modification of any lysine residues.	Attempt to label a different antibody.



Bioconjugation

P-9002-002 — R-PE Antibody Conjugation Kit

Problem	Possible Cause	Recommended Action
Poor HyNic modification of antibody.	Initial antibody concentration is too low.	Concentrate antibody using a diafiltration filter. Use an initial concentration of 2.5- 4 mg/ml antibody with 20-30 molar equivalents of S-HyNic for required labeling of antibody.
	Amine contaminant, e.g. Tris or glycine buffer, present in starting antibody solution.	Thoroughly exchange the antibody buffer by diafiltration, dialysis or desalting column before modification.
R-PE-antibody conjugate has a molecular weight that is much larger than predicted	Due to high modification levels on each antibody a large molecular weight product may be formed.	Decrease modification levels by using lower equivalents of S-HyNic, lower the antibody concentration during the modification reaction.

S-1002 —S-HyNic Linker (DMF Soluble)

Problem	Possible Cause	Recommended Action
Protein was not modified or poorly modified.	Protein has been contaminated with amine containing compounds.	Desalt the protein more thoroughly with a new Zeba Spin column or VivaSpin diafiltration device.
	The concentration of the protein was too low.	Increase the concentration of the protein to >2.0 mg/ml.
S-HyNic was hydrolyzed	Wet or poor quality DMF/DMSO hydrolyzed the NHS ester.	Use a good quality anhydrous DMF/DMSO to solubilize the S-HyNic molecule.

S-1004 — S-4FB Linker (DMF Soluble)

Problem	Possible Cause	Recommended Action
Protein was not modified or poorly modified.	Protein has been contaminated with amine containing compounds.	Desalt the protein more thoroughly with a new Zeba Spin column or VivaSpin diafiltration device.
	The concentration of the protein was too low.	Increase the concentration of the protein to >2.0 mg/ml.
S-4FB was hydrolyzed	Wet or poor quality DMF/DMSO hydrolyzed the NHS ester.	Use a good quality anhydrous DMF/DMSO to solubilize the S-4FB molecule.



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Bioconjugation

S-1008 — Sulfo S-4FB Linker (Water Soluble)

Problem	Possible Cause	Recommended Action
Protein was not modified or poorly modified.	Protein has been contaminated with amine containing compounds.	Desalt the protein more thoroughly with a new Zeba Spin column or VivaSpin diafiltration device.
	The concentration of the protein was too low.	Increase the concentration of the protein to >2.0 mg/ml.
Sulfo S-4FB was hydrolyzed	The buffer hydrolyzed the NHS ester.	Modify the surface, protein or oligo with the linker immediately after dissolving.

S-1009-010 — MHPH (Maleimide HyNic) Linker

Problem	Possible Cause	Recommended Action
Protein was not modified or poorly modified.	Protein has been contaminated with amine containing compounds.	Desalt the protein more thoroughly with a new Zeba Spin column or VivaSpin diafiltration device.
	The concentration of the protein was too low.	Increase the concentration of the protein to >2.0 mg/ml.
	Insufficient number of reduced thiol groups for modification.	Ensure there are sufficient reduced thiol groups.
Sulfo S-4FB was hydrolyzed	The buffer hydrolyzed the NHS ester.	Modify the surface, protein or oligo with the linker immediately after dissolving.

S-1011-010 — Sulfo S-HyNic Linker (Water Soluble)

Problem	Possible Cause	Recommended Action
Protein was not modified or poorly modified.	Protein has been contaminated with amine containing compounds.	Desalt the protein more thoroughly with a new Zeba Spin column or VivaSpin diafiltration device.
	The concentration of the protein was too low.	Increase the concentration of the protein to >2.0 mg/ml.
Sulfo S-HyNic was hydrolyzed	Wet or poor quality DMF/DMSO hydrolyzed the NHS ester	Use a good quality anhydrous DMF/DMSO to solubilize Sulfo-S-HyNic.



Bioconjugation

S-9010-1 — Protein-Protein Conjugation Kit

Problem	Possible Cause	Recommended Action
Poor modification of protein	Initial protein concentration is too low.	Concentrate protein using a diafiltration apparatus to 1-5 mg/ml for efficient modification of protein.
	Insufficient equivalents of linker added.	Use higher equivalents of linker to protein. Up to 30 equivalents can sometimes be added.
	Amine contaminant, e.g. glycine, present in starting biomolecule solution.	Buffer exchange the protein by desalting, dialysis or diafiltration before modification.
	The protein being modified has insufficient amino groups.	Verify lysine content using the NCBI protein database, if possible.
Molar substitution assay readings are out of range	Precipitation of the modified protein on treatment with quantification reagents can lead to spurious reading.	Ensure MSR reactions are clear and free of precipitate before reading.
Precipitation of linker-modified protein	Over-modification with linker.	Decrease equivalents of linker to protein in the modification reaction.
Protein precipitates during conjugation reaction	Conjugation reaction pH may be close to the isoelectric point of the conjugate being formed.	Conjugate at a different pH, higher or lower than PI (but below pH 6.5).

S-9011-1 — Protein-Oligonucleotide Conjugation Kit

Problem	Possible Cause	Recommended Action
Poor HyNic modification of protein	Amine contaminant, e.g. Tris or glycine buffer, present in starting IgG solution.	Thoroughly exchange the protein buffer by diafiltration, dialysis or desalting column before modification.
	Initial protein concentration is too low.	Concentrate protein using a diafiltration filter. Use an initial 2-5 mg/ml for efficient labeling of proteins.
Precipitation of protein on modification	Over modification of the protein.	Spin down precipitate and try to recover any protein left in solution. May require modifying new batch of protein with fewer equivalents.
Protein precipitates during conjugation reaction	Conjugation reaction pH may be close to the isoelectric point of the conjugate being formed.	Conjugate at a different pH (e.g. pH 5, 6, 7 or higher).
Protein-DNA conjugates are degraded	Conjugation reaction contains either single or double stranded nucleases.	Use only molecular grade water (DNase-free) when conjugating DNA to proteins.



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