Version 6 Last updated 7 July 2021

ab270705 5’ Feature Barcode Antibody Conjugation Kit - Lightning-Link® - Oligos 11-20

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

Table of Contents

1. Overview

5' Feature Barcode Antibody Conjugation Kit - Lightning-Link® - Oligos 11-20 (ab270705) enables simple and rapid conjugation of oligonucleotide barcodes to antibodies to enable cell surface protein analysis in Multiomic Cytometry on 10x Genomics Chromium Controller. This kit is compatible with Chromium Single Cell Immune Profiling.

The kit includes 10 distinct oligonucleotide barcodes ready to conjugate to 10 µg of antibody each. The protocol involves only a few steps, requires minimal hands-on time and doesn't require any expertise in antibody conjugation or oligonucleotide sourcing.

Please find antibody buffer considerations and compatibility in the protocol booklet. For incompatible buffers and low antibody concentrations, please see our antibody purification and concentration kits.

For conjugating antibodies with additional distinct oligonucleotide barcodes, please see 5' Feature Barcode Antibody Conjugation Kit - Lightning-Link® - Oligos 1-10 (ab270703) and 5' Feature Barcode Antibody Conjugation Kit - Lightning-Link® - Oligos 21-30 (ab270709). 100 µg conjugation kits are available on-demand for each of these 30 oligonucleotide barcodes. For conjugating more than 30 antibodies with distinct oligonucleotide barcodes, please contact us to discuss your requirements.

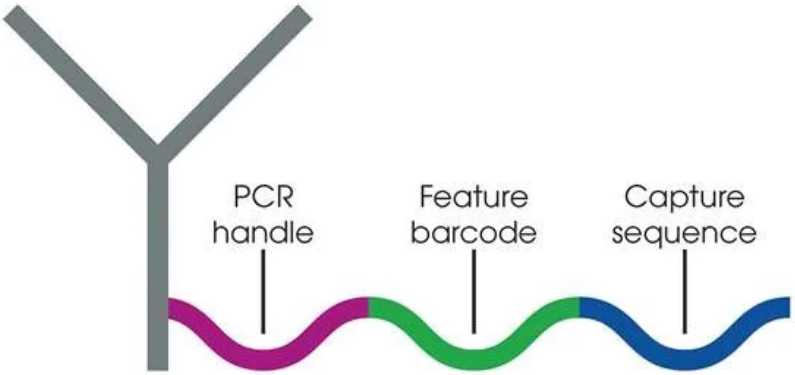

Fig 1. The 5' Feature Barcode Antibody Conjugation Kit - Lightning-Link® includes oligonucleotide barcodes compatible with Chromium Single Cell Immune Profiling from 10X Genomics, which are in the format:

5' CGGAGATGTGTATAAGAGACAGNNNNNNNNN
XXXXXXXXXXXXXXXNNNNNNNNNCCCATATAAGA*A*A 3'

- X represents the nucleotides of the unique barcode.
- N represents a randomly selected A, C, G, or T.
- The symbol * indicates a phosphorothioated bond, this modification renders the internucleotide linkage resistant to nuclease degradation.

2. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

3. Storage and Stability

- Store the kit at -20°C upon receipt.

Lyophilized Lightning-Link® components are hygroscopic.

Kits are intentionally shipped at ambient temperature with silica gel to avoid exposure to moisture. Upon receipt, store the kit frozen and protect from moisture. Before opening the outer container, allow the lyophilized components to reach room temperature to minimize condensation.

4. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

5. Materials Supplied

Item	Quantity	Storage Condition
Barcode Modifier	1 vial	-20°C
Conjugate Resuspension Buffer	1 vial	-20°C
Oligo barcodes 10X5011-10X5020	10 vials	-20°C
Quencher N	1 vial	-20°C
Reagent C	1 vial	-20°C
Reagent S	1 vial	-20°C

6. Technical Hints

- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.

7. Buffer Considerations

– Recommended buffer conditions and components.

Buffer Components	
pH	6.5-8.5
Amine free buffers (e.g. MOPS, MES, HEPES, PBS)	Ok
Non-buffering salts (e.g. sodium chloride)	Ok
Chelating agents (e.g. EDTA)	Ok
Sugars	Ok
Glycerol	Not tested
Thiomersal/ Thimerosal	Not recommended
Merthiolate	Not recommended
Sodium azide	≤ 0.25%
BSA	Not recommended
Gelatin	Not recommended
Tris	Not recommended
Glycine	Not recommended
ProClin 300	Not recommended
Borate buffer	Not tested
Nucleophilic components (e.g. amino acids, ethanolamine, mercaptoethanol or DTT)	Not recommended

ΔNote: Compounds above marked 'not tested' yet are expected to be tolerated but no confirmatory data is available at this time. Please note that this table refers to expected impact in conjugation. You should also give due consideration to effects of these substances in your application, especially if the optional purification step is not required.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- Antibodies should be 1 mg/mL in a suitable buffer (See Section 7 for compatible substances). Antibodies that are <1 mg/mL should ideally be concentrated,

8.1 Optional: Reagent S will need to be placed in warm water (not warmer than 40°C) for 15 minutes and mixed regularly. If the sample does not dissolve completely, spin the tube in a bench top micro-centrifuge at a recommended maximum speed of 13,000g for 1 minute, and use the supernatant. Store at room temperature after initial thawing/warming.

8.2 Dilute your antibody to 1 mg/mL (See Section 7 for buffer compatibility) and add 1 µL of Barcode Modifier reagent to 10 µL of antibody.

8.3 Remove the screw cap from a vial of Oligo barcodes and pipette 11 µL of antibody/Barcode Modifier mix directly onto the lyophilized material. Resuspend gently by withdrawing and re-dispensing the liquid until the powder dissolves.

8.4 Place the cap back on the vial and leave the vial standing at room temperature (20-25°C) overnight. It is convenient to set up reactions late in the day though the exact set-up time is not critical.

8.5 At the end of the overnight incubation, add 1 µL of Quencher N and leave at room temperature for 15 minutes. The conjugate is now ready.

Δ Note: There are two optional steps – (i) purification of the conjugate to remove free oligo and (ii) SDS gel analysis of a small aliquot of the conjugate. Either or both steps may be appropriate, and these are explained in Sections 11 and 12.

9. Storage of Conjugates

- For any new conjugate, initial storage at 4°C is recommended. A preservative may be desirable for long-term storage. Other storage conditions (e.g. frozen at -70°C or at -20°C with 50% glycerol) may also be satisfactory. The best conditions for any new conjugate must be determined by experimentation. You should also take account of how the conjugate will be used and avoid adding substances that will only need to be removed later, resulting in inevitable loss of conjugate.

10. Varying the degree of labeling

- Antibodies that are too concentrated can be diluted in an amine-free buffer (see Section 7) to 1 mg/mL. In most cases you will therefore be adding 11 µL (10 µL + 1 µL, from step 8.2) to the oligo reaction vial.
- As the required degree of labeling may be application dependent, there are circumstances where one might elect to vary the mass or concentration of antibody, within limits, to alter the average number of oligos per antibody and hence to fine tune conjugate performance. The recommended range for exploration is 10 – 20 µg of antibody in 10 – 20 µL volume (or 11 – 22 µL volume with Barcode Modifier).
- Some examples showing the effect of changing reaction conditions are given in Section 12.

11. Conjugate Clean Up (Optional)

– If you intend to do SDS-PAGE, remove 1 µL of conjugate before carrying out clean-up, as the clean-up reagents will interfere with gel analysis.

11.1 Make sure that Reagent S is fully in solution.

11.2 Reconstitute Reagent C with 500 µL of pure water and pipette up and down 2-3 times (or gently swirl) to dissolve the powder.

11.3 Add 40 µL of Reagent C to the ~10 µL of conjugate to be cleaned-up and transfer the mixture to an Eppendorf tube. (Ideally the tube should have high transparency).

11.4 Add 50 µL of Reagent S and mix gently. Wait 3 mins. The solution should be slightly turbid. (This may be easier to see if you compare your tube to one comprising 50 µL water + 50 µL Reagent S).

11.5 Spin the tube at 15,000g in a microfuge for 5 minutes. Position the Eppendorf tube in the centrifuge in such a manner that you know where your pellet will be located.

11.6 Using a P200 (not a P1000), very carefully remove (and save) the supernatant without dislodging the pellet. If you accidently suck up some of the pellet, eject the material and spin for a few seconds to compact the pellet again.
Δ Note: keep the supernatant until successful recovery of the conjugate has been confirmed later in the procedure.
It is important to remove as much supernatant as possible. As some liquid always remains on the sides of the tube, you can spin the tube again for a few seconds and recover more supernatant preferably using a P20. The more of the supernatant that is removed, the greater the efficiency of removal of any unconjugated oligo.

11.7 Once you are satisfied the tube is substantially free of surplus liquid, resuspend the pellet in 50 µL of Conjugate Resuspension Buffer.

11.8 Repeat the procedure in steps 11.4-11.6.

11.9 The final pellet should be resuspended in a buffer of your choice, ideally compatible with your intended application. Alternatively, the Conjugate Resuspension Buffer in the kit may be used.

11.10 Store the cleaned-up conjugate at 4°C.

12. SDS-PAGE (Optional)

– SDS-PAGE may be used to confirm successful conjugation. To carry out SDS gel analysis, remove 1 µL of conjugate prior to optional clean-up and add to 10 µL of either reducing or non-reducing sample buffer. Run the samples on a gradient gel (a 4-12% gel is ideal).

12.1 Reducing gels have the advantage that antibody heavy (H) and light (L) chains migrate some distance in the gel and the band shifts caused by the attachment of oligos are quite pronounced. Reducing gels also provide an indication of how many oligos have been attached to the H and L chains. Please note that this number will be in a range, not a single number. Some heavy and light chains will also remain unlabeled, even if an IgG has reacted with several oligos.

12.2 On non-reducing gels, the four antibody chains migrate as a single unit and an IgG molecule with just one oligo attached will always exhibit a band shift. (On a reducing gel one chain would exhibit a band shift and three would not). A non-reducing gel is therefore very useful for demonstrating the absence of unlabeled antibodies in conjugates, however, the resolution of the various bands is less than on a reducing gel and it is particularly important not to overload non-reducing gels.

12.3 An example is shown in Fig. 2 with a polyclonal antibody. The gel has been deliberately overloaded (~4 µg of antibody conjugate and IgG) to illustrate the band patterns more clearly, especially the quantitatively minor bands in the reducing half of the gel (Fig. 2, Tracks 3 and 4). i.e. those with two or more oligos attached.

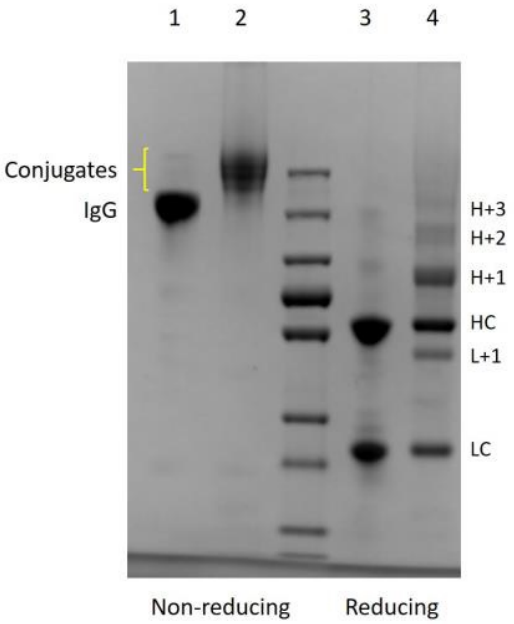

Fig 2.

12.4 On the non-reducing half of the gel (Tracks 1 and 2), the block of overlapping conjugate bands (Track 2) clearly has lower mobility than the reference IgG, indicating that no unlabeled antibodies are present in the conjugate. Sometimes discrete conjugate bands will be seen on non-reducing gels and this is dependent on the type of antibody, quality of the gradient gel and the amount of sample loaded. Irrespective of the resolution within the conjugate bands, one may deduce from the extent of the band shift compared with an IgG reference band whether any unlabeled antibodies are present in the conjugate.

12.5 Discrete conjugate bands are more commonly seen with monoclonal antibodies and with lower loadings than those used above (see Fig. 3 below).

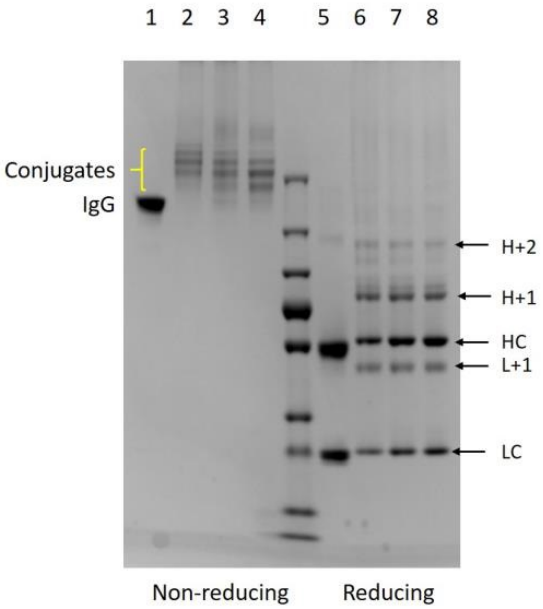

Fig 3.

12.6 Tracks 2 and 6 were loaded with 2 µg of conjugate produced using the preferred procedure (10 µg of antibody added in 11 µL final volume) under non-reducing or reducing conditions. At this lower loading, the H+2 band is still visible but H+3 (not labeled on Fig. 3) is faint. On the non-reducing gel, the number of oligos attached to IgGs is readily determined. Further commentary and details of the other samples (Tracks 3, 4, 7 and 8) are given below. A magnified view of the IgG conjugates in Fig. 3 is shown in Fig. 4 below.

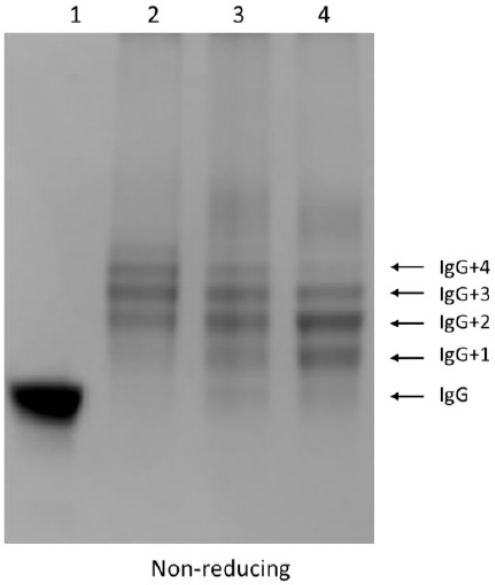

Fig 4.

12.7 In Fig. 4, track 2 shows no free IgG and very little IgG with 1 oligo attached. Mostly there are 2-5 oligos per IgG (IgG+5 is not marked by an arrow above). In tracks 3 and 4, the conjugation reactions comprised, respectively, 20 µg in 10 µL and 20 µg in 20 µL. Not surprisingly, the labeling density changes and there are 1-4 oligos per IgG (but mainly 2-3 oligos) in track 3. In track 4, most conjugates have 1 or 2 oligos. As shown, there are smaller quantities of unlabeled IgG as the average oligo density is reduced.

12.8 The differences among the samples under reducing conditions (Tracks 6, 7 and 8, Fig. 3) are less pronounced and the main observation is increasing H or L chain intensity as conjugation efficiency is reduced. For each application, it may be necessary to determine the gel profile that is associated with best assay performance, and non-reducing gels are generally more useful.

12.9 If only reducing gel data are available, there are still pointers that help with the assessment of conjugation efficiency and the likelihood of there being no unlabeled antibodies. One would expect to see bands corresponding to H, H+1, H+2 and H+3. L+1 will be clearly visible and L+2 will be present, although partially obscured by H+1. The H+3 bands are easier to see if a large amount of sample is applied, but this is not always desirable (see below).

12.10 Another useful approach is to run a matched amount of unlabeled antibody. A reduction in the intensity of the H and L chains in the conjugate sample of around 50% or more (because of the shifting of some of the H and L chains), provides a strong indication, on probability grounds, that most IgG molecules will have at least one oligo attached.

12.11 Finally, with 10 µg scale conjugation, one would probably be reluctant to run more than 1 µL of conjugate routinely on gels. A typical profile for 1 µL (from an 11 µL scale reaction) under both non-reducing and reducing conditions is shown below in Figure 5. You may prefer to run 1 µL of sample in just one of these conditions.

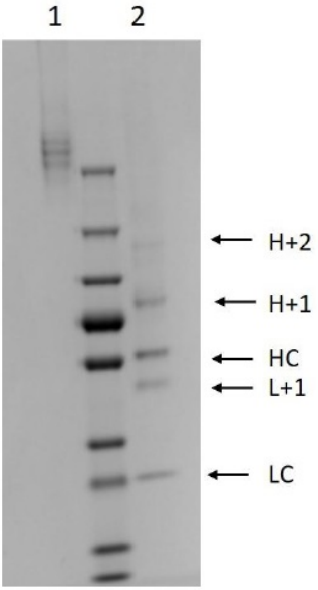

Fig 5.

12.12 The non-reduced sample (Track 1) shows clearly that all antibodies are conjugated (in this case mostly with 1-3 oligos). Following reduction, L+1, H+1 and H+2 are clearly visible. H+3 may be absent when only 1 µL of conjugate is analyzed (~0.9 µg) in reducing conditions.

13. Troubleshooting

My gel has no conjugate bands. What happened?

An absence of conjugate bands almost with exception indicates that the antibody contains interfering substances. Things to watch out for (in addition to substances discussed in Section 7) are whether the antibody is provided as tissue culture supernatant or ascites fluid, each of which must be purified before conjugation. It is also important to understand if the mass of protein stated on the vial relates to antibody or to antibody plus other substances e.g. BSA. Finally, you should be wary of antibodies that are sold as '100 tests' or '100 µL', especially if other details are lacking. In these formats the antibody has usually been heavily diluted.

My antibody is too dilute - what should I do?

Ideally you should concentrate the sample using spin filters. As there will be some loss of antibody, you will need considerably more than 10 µg to carry out this procedure. Thus, if you only have 10 µg and the concentration is low (e.g. >0.5 mg/mL and <1 mg/mL), it may be better (though not ideal) to perform conjugation under suboptimal conditions. Of course, this could negatively impact assay performance.

Do I need to clean up my conjugate?

As there are many different applications only some general guidelines can be given.

If free oligo is likely to be problematic, you will need to clean up the conjugate.

However, if the antibody binds to a surface in the application of interest, it may be possible to wash away any free oligo prior to hybridization/amplification and avoid the conjugate clean-up steps in Section 11.

Section 11 incorporates two clean-up steps, each greatly reducing the level of free oligo. In some applications one clean-up step may be enough (in which case step 8 in Section 11 would be omitted).

Ultimately, you may need to test conjugate with and without clean-up steps to determine if some of the processing steps can be avoided.

14. Appendix

ab ID	Oligo Code	Unique Barcode Sequence	Full Oligo Sequence (5' to 3')
ab270703 5' Feature Barcode Antibody Conjugation Kit Lightning-Link® Oligos 1-10	Oligo 10XS001	GTACTTGATTAGCCT	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNGTACTTGATTAGCCTNNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS002	TCCGAGTCCGTCGCG	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNTCCGAGTCCGTCGCGNNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS003	GAGTTGCGAAGTTCT	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNGAGTTGCGAAGTTCTNNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS004	TGCTAACACAACGAG	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNTGCTAACACAACGAGNNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS005	CAAGCATGTAGGAGT	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNCAAGCATGTAGGAGTNNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS006	CCGCGAATCAGCCGC	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNCCGCGAATCAGCCGCNNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS007	CTTCTACAACAATGG	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNCTTCTACAACAATGGNNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS008	CCTAATGGTCCAGAC	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNCCTAATGGTCCAGACNNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS009	GTGGTAAGCAGACGC	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNGTGGTAAGCAGACGCNNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS010	CCGGTTCTGGCACAG	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNCCGGTTCTGGCACAGNNNNNNNNNNCCCATATAAGA*A*A
ab270705 5' Feature Barcode Antibody Conjugation Kit Lightning-Link® Oligos 11-20	Oligo 10XS011	ATGTTATGCGTCGTG	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNATGTTATGCGTCGTGNNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS012	GGTGATTACTGGAGA	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNGGTGATTACTGGAGANNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS013	GCGGCGAAGAAGCTA	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNGCGGCGAAGAAGCTANNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS014	ATAGCTCTGGCAACG	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNATAGCTCTGGCAACGNNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS015	AAGAACCTCTCACAT	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNAAGAACCTCTCACATNNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS016	TTCATTCGCATCATC	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNTTCATTCGCATCATCNNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS017	TATTACAAGCGCAAT	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNTATTACAAGCGCAATNNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS018	CATTGTCGACCGCGA	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNCATTGTCGACCGCGANNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS019	GCACAGTCCGCAATC	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNGCACAGTCCGCAATCNNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS020	TAATACCGGAGCCGA	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNTAATACCGGAGCCGANNNNNNNNNNCCCATATAAGA*A*A
ab270709 5' Feature Barcode Antibody Conjugation Kit Lightning-Link® Oligos 21-30	Oligo 10XS021	AGGAACCGACACCTA	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNAGGAACCGACACCTANNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS022	TCTGCACCTTGCACG	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNTCTGCACCTTGCACGNNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS023	ACATGATATATTCAC	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNACATGATATATTCACNNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS024	TTAGCCTACGATACT	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNTTAGCCTACGATACTNNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS025	GCAGAATGAGCGTCA	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNGCAGAATGAGCGTCANNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS026	ACCTGGCAAGCTAGT	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNACCTGGCAAGCTAGTNNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS027	GACTACATAGGCTCC	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNGACTACATAGGCTCCNNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS028	GTGACATCGGCGCTA	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNGTGACATCGGCGCTANNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS029	ATTGAAGCGGCGCTT	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNATTGAAGCGGCGCTTNNNNNNNNNNCCCATATAAGA*A*A
	Oligo 10XS030	CGCTACGCTCCAATA	CGGAGATGTGTATAAGAGACAGNNNNNNNNNNNNCGCTACGCTCCAATANNNNNNNNNNCCCATATAAGA*A*A

15. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)