



Flow Cytometry Analysis of BD™ AbSeq Ab-Oligo and Sample Tag Expression Protocol

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The unsupported protocol described herein may not have been validated and is provided as is and without any warranty of any kind. Any use of this protocol is at the risk of the user, and users are responsible for performing validation as appropriate. BD reserves the right to change specifications at any time. Information in the protocol is subject to change without notice. BD assumes no responsibility for any errors or omissions. In no event shall BD be liable for any damages in connection with or arising from the use of this protocol.

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Introduction

Overview and intended use

The purpose of this document is to provide detailed instructions on how to use single-color flow cytometry to analyze expression of oligonucleotide-conjugated antibodies with a poly(A) tail. This protocol allows rapid analysis of the expression of protein targets in the sample of interest when labelling cells with Sample Tags from the BD™ Single-Cell Multiplexing Kit or with BD AbSeq Ab-Oligos.

Safety symbols

The following table lists the safety symbols used in this protocol to alert you to potential hazards.

Symbol	Meaning
	Caution. Indicates the need for the user to consult the instructions for use for important cautionary information, such as warnings and precautions that cannot, for a variety of reasons, be presented on the device itself.
	Biological hazard. All surfaces that come in contact with biological specimens can transmit potentially fatal disease. Use universal precautions when cleaning surfaces. Wear suitable protective clothing, eyewear, and gloves.

Safety data sheets

Before handling chemicals, read and understand the appropriate safety data sheets (SDSs). To obtain SDSs for chemicals ordered from BD Biosciences, go to regdocs.bd.com, or contact BD Biosciences technical support at researchapplications@bd.com.

Required and recommended materials

Required kits

Use the required kits that are specified in the protocol that you use to label cells with Sample Tags and/or BD AbSeq Ab-Oligos. See [Labelling cells on page 4](#).

Required reagents

Material	Supplier	Catalog no.
20,000–1 million cells labelled with Sample Tags and/or BD AbSeq Ab-Oligos	—	—
BD Stain Buffer (FBS)	BD Biosciences	554656
DNA Suspension Buffer	Teknova	T0221
Flow proxy oligo (stock concentration of 100 µM oligo in 10 mM Tris, 0.1 mM EDTA, pH 8.0): 5' TTTTTTTTTTTTTTTTTTTT-AF647 3'	Any DNA supplier	—

Required consumables and equipment

Material	Supplier	Catalog no.
Falcon® tubes, 5 mL Round Bottom Polystyrene Test Tube	Corning	352054
DNA LoBind Tubes, 1.5 mL	Eppendorf	0030108051
Centrifuge and rotor for 5 mL Falcon tubes	Major supplier	—
Pipettes (P10, P20, P200, P1000)	Major supplier	—
Low retention filtered pipette tips: 10 µL, 10 µL, 200 µL, 1,000 µL	Major supplier	—
Flow cytometer	Major supplier	—

Labelling cells

Follow the appropriate protocol to label cells with Sample Tags and/or BD AbSeq Ab-Oligos. Use the appropriate reagent kits specified in the protocol:

- *Preparing Single Cell Suspensions Protocol* (Doc ID: 210964)
- *Single Cell Labelling with the BD™ Single-Cell Multiplexing Kit Protocol* (Doc ID: 210970)
- *Single Cell Labelling with BD™ AbSeq Ab-Oligos Protocol* (Doc ID: 214394)
- *Single Cell Labelling with the BD™ Single-Cell Multiplexing Kit and BD™ AbSeq Ab-Oligos Protocol* (Doc ID: 214419)

Genomics technical publications are available for download from the BD Genomics Resource Library at bd.com/genomics-resources.

Secondary labelling with the flow proxy oligo

- 1 Add 3.75 µL of 100 µM flow proxy oligo to 96.25 µL DNA Suspension Buffer to dilute the stock concentration of flow proxy oligo from 100 µM to 3.75 µM.
- 2 Follow the appropriate cell labelling protocol to label 20,000–1 million cells, but at the final step, resuspend labelled cells in 100 µL of BD Stain Buffer (FBS) rather than 100 µL of Sample Buffer. For cell labelling protocols, see [Labelling cells](#).
- 3 Pipet 1 µL of 3.75 µM flow proxy oligo into each tube containing 100 µL of labelled cells in BD Stain Buffer (FBS). Pipet-mix 10 times.
- 4 Incubate the cells at room temperature (15°C to 25°C), protected from light, for 20 minutes.
- 5 Pipet-mix the cells, and then transfer each flow-proxy-oligo-labelled cell suspension to a 5 mL polystyrene Falcon tube, add 2 mL of BD Stain Buffer (FBS) to each tube, and pipet-mix.
- 6 Centrifuge the tube at 400 × g for 5 minutes.
- 7 Uncap each tube, and invert to decant supernatant into the biohazardous waste container. Keep the tube inverted and gently blot on a lint-free wiper to remove residual supernatant from tube rim.
- 8 Add 2 mL of BD Stain Buffer (FBS) to each tube, and resuspend by pipet-mixing.
- 9 Centrifuge the tube at 400 × g for 5 minutes.
- 10 Uncap each tube, and invert to decant supernatant into the biohazardous waste container. Keep the tube inverted and gently blot on a lint-free wiper to remove residual supernatant from tube rim.
- 11 Resuspend each pellet in 350 µL of BD Stain Buffer (FBS).

Analyzing cell labelling by flow cytometry

The samples are now ready to be analyzed on a flow cytometer capable of measuring Alexa Fluor® 647. The flow proxy oligo is conjugated to Alexa Fluor® 647, which has an excitation wavelength of 650 nm and emission wavelength of 665 nm.

Safety overview

Chemical safety

Requirements

- Read and comprehend all SDSs by chemical manufacturers before you use, store, or handle any chemicals or hazardous materials.
- Wear personal protective equipment (gloves, safety glasses, fully enclosed shoes, lab coats) when handling chemicals.
- Do not inhale fumes from chemicals. Use adequate ventilation, and return caps to bottles immediately after use.
- Check regularly for chemical spills or leaks. Follow SDS recommendations for cleaning up spills or leaks.

Waste

Collect and dispose of all waste in the appropriate waste collection container using proper precautions and according to local safety regulations.

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