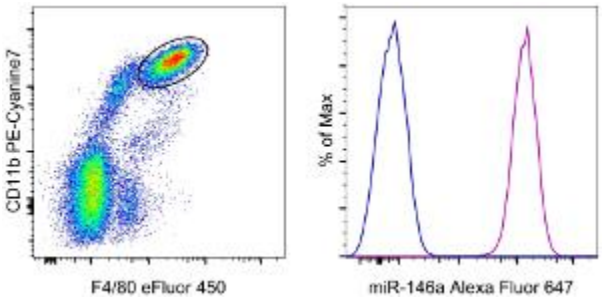
PrimeFlow® microRNA Pretreatment Buffer

Catalog Number: 88-18006

RUO: For Research Use Only. Not for use in diagnostic procedures.



C57Bl/6 mouse peritoneal exudate cells were incubated with Anti-Mouse CD16/CD32 Purified (cat. 14-0161) and Normal Mouse Serum (cat. 24-5544) to block Fc receptors, and then surface stained with Anti-Mouse F4/80 Antigen eFluor® 450 (cat. 48-4801) and Anti-Mouse CD11b PE-Cyanine7 (cat. 25-0112) (right). Subsequently, the cells were fixed with the PrimeFlow® microRNA Pretreatment Buffer (cat. 88-18006), then hybridized to Type 1 Mouse miR-146a Alexa Fluor® 647 Target Probe Set (cat. VM1-10253) (purple histogram) using the PrimeFlow® RNA Assay (cat. 88-18005). The no target probe control is shown in the blue histogram. F4/80 and CD11b double-positive cells were used for analysis.

Product Information

Contents: PrimeFlow® microRNA Pretreatment Buffer

REF **Catalog Number:** 88-18006

Temperature Limitation: Store at 2-8°C.

LOT **Batch Code:** Refer to vial

Use By: Refer to vial

Contains <10% formaldehyde

Description

This PrimeFlow® microRNA Pretreatment Buffer is designed to be used in combination with the PrimeFlow® RNA Assay (cat. no. 88-18005) for the optimal detection of microRNA and other small RNA by flow cytometry. Because microRNA molecules have a limited number of target probe binding sites per molecule, we recommend using Type 1, Alexa Fluor® 647 or Type 10, Alexa Fluor® 568 Target Probe sets for optimal sensitivity. This buffer may also be used in combination with the PrimeFlow® Fixation/Permeabilization Buffer Set (cat. no. 88-17000) to evaluate antibody performance for intracellular targets. Follow the protocol that is attached to this data sheet. Please note that additional changes in antibody performance may be observed after the hybridization steps of the PrimeFlow RNA Assay.

The PrimeFlow® RNA Assay and Target Probe Sets for genes of interest are sold separately.

Components

PrimeFlow® microRNA Pretreatment Concentrate (4X): cat. 00-16009, 25 mL, store at 2-8°C
PrimeFlow® microRNA Pretreatment Diluent: cat. 00-16008, 75 mL, store at 2-8°C

Applications Reported

This PrimeFlow® microRNA Pretreatment Buffer has been reported for use in flow cytometry.

Applications Tested

This PrimeFlow® microRNA Pretreatment Buffer has been tested by intracellular staining and flow cytometric analysis using the attached protocol. Please refer to the PrimeFlow® RNA Assay (cat. 88-18005) User Manual for full details on assay specifications and guidelines.

Related Products

14-0161 CD16/CD32 Monoclonal Antibody (93), eBioscience™ TDS DISABLED: ABMAINT SKU (93)
24-5544 Normal Mouse Serum Control, eBioscience™ TDS DISABLED: ABMAINT SKU
25-0112 CD11b Monoclonal Antibody (M1/70), PE-Cyanine7, eBioscience™ TDS DISABLED: ABMAINT SKU (M1/70)
44-17005 PrimeFlow® 96-well plate

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • thermofisher.com/ebioscience • info@ebioscience.com

PrimeFlow® microRNA Pretreatment Buffer

Catalog Number: 88-18006
RUO: For Research Use Only. Not for use in diagnostic procedures.

48-4801 F4/80 Monoclonal Antibody (BM8), eFluor 450, eBioscience™ TDS DISABLED: ABMAINT SKU (BM8)
88-17000 PrimeFlow® Fixation/Permeabilization Buffer Set
88-18005 PrimeFlow® RNA Assay

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PrimeFlow™ RNA Assay with microRNA Pretreatment Protocol

Catalog Number 88-18006

Introduction

This protocol describes the use of the PrimeFlow™ microRNA Pretreatment Buffer with the PrimeFlow™ RNA Assay to enhance detection of microRNA. The Pretreatment Buffer improves the retention of some small RNA targets resulting in higher signal and better sensitivity. The PrimeFlow™ RNA Assay combines the power of the branched DNA technology with the single cell resolution of flow cytometry to allow simultaneous detection of up to four microRNA/RNA targets in combination with cell surface and intracellular staining using fluorochrome-conjugated antibodies to allow further discrimination of specific cell subpopulations.

General Notes

Note: Refer to the *PrimeFlow™ RNA Assay User Manual* for full details on assay specifications and guidelines.

Guidelines for PrimeFLow™ microRNA Pretreatment Buffer

1. Due to the short length of microRNA, the signal generated per molecule of microRNA is expected to be significantly lower than mRNA. Therefore, it is highly recommended to use Type 1, Alexa Fluor™ 647, or Type 10, Alexa Fluor™ 568, for detection of microRNA for maximal sensitivity.
2. The PrimeFlow™ microRNA Pretreatment Buffer may have a negative impact on the staining of some intracellular proteins. Refer to Table 1 of this protocol for relative performance of selected antibodies, or contact Technical Support for more information. It is highly recommended to evaluate antibody staining before starting a comprehensive experiment.
3. If intracellular antibody staining is critical but impaired by the PrimeFlow™ microRNA Pretreatment Buffer, it may be possible to use the standard PrimeFlow™ RNA Assay and protocol to allow the combination of intracellular staining and detection of microRNA. Some decrease in the sensitivity of the detection of microRNA may be observed. Contact Technical Support for more information.

Materials Included

- PrimeFlow™ microRNA Pretreatment Concentrate (4X) (cat. no. 00-16009), 25 mL, store at 2–8°C

  Note: Contains formaldehyde, which is a poison and irritant. See Safety Data Sheet for complete information.

- PrimeFlow™ microRNA Pretreatment Diluent (cat. no. 00-16008), 75 mL, store at 2–8°C

Materials required, but not included

- PrimeFlow™ RNA Assay Kit (cat. no. 88-18005)
- Flow Cytometry Staining Buffer (cat. no. 00-4222)
- Fluorochrome-conjugated antibodies, as needed
- Fixable Viability Dye, as needed
- Phosphate-buffered saline (PBS)
- Target Probe sets, as needed
- 12 x 75 mm polystyrene tubes (e.g., Corning™, cat. no. 352008)
- [OPTIONAL] 96-well, v-bottom polystyrene plate (cat. no. 44-17005)

Note: Refer to Protocol B for a modified protocol to use 96-well plates.

Instruments and equipment

Refer to Appendix 2 of the *PrimeFlow™ RNA Assay User Manual*.

Experiment Duration

Day 1 (~6–8 hours)

- **Antibody staining**
- **microRNA Pretreatment**
- **Fixation and permeabilization**
- **Target probe hybridization**

Day 2 (~6 hours)

- **Signal amplification**
- **Flow cytometric analysis**

Protocol A: Prime Flow RNA Assay with microRNA Pretreatment using 1.5-mL tubes

Note: This protocol is validated based on the use of a polystyrene 96-well v-bottom plate (cat. no. 44-17005); polystyrene u-bottom plates may also be used. To discard supernatant from the wells, the plate may be inverted, using a single motion with adequate force, and then gently blotted on a paper towel. Alternatively, aspiration may be used, being careful to not disrupt the pellet. The residual volume inside each well should not exceed 10 µL.

Note: Flat-bottom plates are not recommended for use with this protocol.

Day 1: Antibody staining, pretreatment, fixation, and permeabilization

1. Pre-warm PrimeFlow™ RNA Wash Buffer to room temperature. This buffer will first be used in Step 19.

   Note: Some precipitation in the Wash Buffer may occur. After warming to room temperature, an aliquot for the volume needed for the day may be prepared and allowed to settle or briefly centrifuged to remove precipitation. Do not repeatedly centrifuge the Wash Buffer to remove precipitation.

2. Aliquot 1–5 x 106 cells in 100 µL of Flow Cytometry Staining Buffer per well.

   Note: To use whole blood, it is necessary to pre-lyse the red blood cells before beginning the assay using the 10X RBC Lysis Buffer (cat. no. 00-4300). Please see the product datasheet or Best Protocols: Red Blood Cell Lysis Protocol, Protocol A for detailed instructions for use. Alternatively, perform Steps 2–16 in bulk (refer to Protocol A, Steps 2–15, to maintain the optimal sample to reagent ratio).

3. In 12 x 75 mm polystyrene tubes or in a separate 96-well plate, prepare single-color compensation control samples using the PrimeFlow™ Compensation Kit and any experimental antibodies being used. Refer to Appendix 3 of the *PrimeFlow™ RNA Assay User Manual* for detailed instructions.

   Store compensation control samples in the dark at 2–8°C in IC Fixation Buffer by resuspending the stained UltraComp eBeads in 100 µL of Flow Cytometry Staining Buffer then add 100 µL of IC Fixation Buffer and mix gently.

4. Surface stain cells with fluorochrome-conjugated antibodies at their optimal concentration for 30 minutes at 2–8°C.

   Note: Please see Best Protocols: Staining Cell Surface Antigens for Flow Cytometry: Protocol A: Cell Suspensions for detailed instructions for staining. If no surface staining is desired, skip this step and proceed to Step 6.

   Note: Use antibodies that are conjugated to approved fluorochromes only (see General notes, Fluorochrome compatibility section in the *PrimeFlow™ RNA Assay User Manual*).

   Note: Cells may be stained with a Fixable Viability Dye before or after surface staining (see Best Protocols: Viability Staining Protocol, Protocol C for detailed instructions).

   Note: Staining for some surface markers may be done after fixation and permeabilization. Please see the Antibody Clone Performance Following Fixation/Permeabilization table on our website and refer to the column for "After IC Fixation and Perm Wash" to determine if the antibody clone will recognize a fixed epitope. If you prefer to stain after fixation, skip this step and proceed to Step 6.

5. Spin down at 500 x g for 4 minutes at 2–8°C, then discard supernatant.

6. Add 200 µL of Flow Cytometry Staining Buffer to each sample, pipet to mix, and spin down at 500 x g for 4 minutes at 2–8°C, then discard supernatant and resuspend cells in the residual volume by vortexing gently.

7. Prepare 1X PrimeFlow™ microRNA Pretreatment Buffer by combining 50 µL of PrimeFlow™ microRNA Pretreatment Concentrate (4X) with 150 µL of PrimeFlow™ microRNA Pretreatment Diluent per sample. Mix gently by inverting. Protect this working solution from light and store at room temperature until ready for use.

   Note: You will need 200 µL of this buffer per sample. Prepare this buffer in bulk to accommodate all samples. For example, for 10 samples, combine 0.5 mL of PrimeFlow™ microRNA Pretreatment Concentrate (4X) with 1.5 mL of PrimeFlow™ microRNA Pretreatment Diluent. Avoid vortexing or vigorously shaking this buffer. This buffer should be prepared fresh. Dispose of any unused buffer.

8. Add 200 µL of 1X PrimeFlow™ microRNA Pretreatment Buffer to each sample and pipet to mix. Incubate for 15 minutes in the dark at room temperature.

9. Spin down at 1,000 x g for 4 minutes at 2–8°C. Discard supernatant and resuspend cells in the residual volume by vortexing gently.

10. Add 200 µL of 1X PBS to each sample, and pipet to mix. Spin down at 1,000 x g for 4 minutes at 2–8°C. Discard supernatant and resuspend cells in the residual volume by vortexing gently.

11. Prepare Fixation Buffer 1 by mixing together equal parts of PrimeFlow™ RNA Fixation Buffer 1A and PrimeFlow™ RNA Fixation Buffer 1B. Mix gently by inverting.

Note: You will need 200 µL of this buffer per well. Prepare this buffer in bulk to accommodate all samples. Avoid vortexing or vigorously shaking this buffer. This buffer should be prepared fresh. Dispose of any unused buffer.

12. Add 200 µL of prepared PrimeFlow™ RNA Fixation Buffer 1 to each sample and pipet to mix. Incubate for 30 minutes at 2–8°C.
13. Spin down at 1,000 x g for 4 minutes at 2–8°C, then discard supernatant and resuspend cells in the residual volume by vortexing gently.
14. Prepare 1X PrimeFlow™ RNA Permeabilization Buffer with RNase Inhibitors by diluting PrimeFlow™ RNA Permeabilization Buffer (10X) to 1X with RNase-free water. Then add RNase Inhibitors (100X) at a 1/100 dilution. Mix gently by inverting. Keep at 2–8°C.

    Note: You will need 700 µL of this buffer per sample. Prepare this buffer in bulk to accommodate all samples. Avoid vortexing or vigorously shaking this buffer. This buffer should be prepared fresh. Dispose of any unused buffer.

15. Add 200 µL of 1X PrimeFlow™ RNA Permeabilization Buffer with RNase Inhibitors to each sample, pipet to mix, and spin down at 1,000 x g for 4 minutes at 2–8°C, then discard supernatant and resuspend cells in the residual volume by vortexing gently.
16. Repeat Step 15.

    Note: Whole blood samples being prepared in bulk should be transferred into a 96-well plate before proceeding to Step 17.

17. Intracellularly stain cells with fluorochrome-conjugated antibodies at their optimal concentration in the 1X PrimeFlow™ RNA Permeabilization Buffer with RNase Inhibitors for 30 minutes at 2–8°C.

    Note: If intracellular staining is not desired, skip this step and proceed to Step 19.

    Note: Staining for some surface markers may be done after fixation and permeabilization. Please see the Antibody Clone Performance Following Fixation/Permeabilization table on our website and refer to the column for "After IC Fixation and Perm Wash" to determine if the antibody clone will recognize a fixed epitope. If you prefer to stain after fixation, skip this step and proceed to Step 6.

    Note: Use antibodies that are conjugated to approved fluorochromes only (see General notes, Fluorochrome compatibility section in the PrimeFlow™ RNA Assay User Manual).

18. Add 200 µL of 1X PrimeFlow™ RNA Permeabilization Buffer with RNase Inhibitors to each sample, pipet to mix, and spin down at 1,000 x g for 4 minutes at 2–8°C, then discard supernatant and resuspend cells in the residual volume by vortexing gently.
19. Prepare 1X PrimeFlow™ RNA Fixation Buffer 2 by combining 25 µL of PrimeFlow™ RNA Fixation Buffer 2 (8X) with 175 µL of PrimeFlow™ RNA Wash Buffer per well. Mix gently by inverting.

    Note: You will need 200 µL of this buffer per well. Prepare this buffer in bulk to accommodate all samples. For example, for 10 samples, combine 250 µL of PrimeFlow™ RNA Fixation Buffer 2 (8X) with 1,750 µL of PrimeFlow™ RNA Wash Buffer. Avoid vortexing or vigorously shaking this buffer.

    This buffer should be prepared fresh. Dispose of any unused buffer.

20. Add 200 µL of 1X PrimeFlow™ RNA Fixation Buffer 2 to each well and pipet to mix. Incubate for 60 minutes in the dark at room temperature.

    Note: It is important to fix the samples at room temperature. Do not perform this step on ice.

21. [OPTIONAL] Cells may be stored in 1X PrimeFlow™ RNA Fixation Buffer 2 overnight in the dark at 2–8°C, instead of incubating for 60 minutes at room temperature (i.e., skip Step 20).
22. Spin down at 1,000 x g for 4 minutes at room temperature, then discard supernatant and resuspend cells in the residual volume by vortexing gently.
23. Add 200 µL of PrimeFlow™ RNA Wash Buffer to each well, pipet to mix, and spin down at 1,000 x g for 4 minutes at room temperature, then discard supernatant and resuspend cells in the residual volume by vortexing gently.
24. Repeat Step 23.
25. [OPTIONAL] Cells may be stored in PrimeFlow™ RNA Wash Buffer with RNase Inhibitors overnight in the dark at 2–8°C with the lid on the plate. To do so, add RNase Inhibitors (100X) to PrimeFlow™ RNA Wash Buffer at a 1/100 dilution and use in Step 24.

    Note: You will need 100 µL of this buffer per well. Prepare this buffer in bulk to accommodate all samples. This buffer should be prepared fresh. Dispose of any unused buffer.

Day 1: Target probe hybridization

Note: It is critical that the residual volume after all washes does not exceed 10 µL.

Note: Diluted Target Probes should be pipetted directly into the cell suspension, and samples should be mixed well before incubating. The total volume for Target Probe hybridization should be 200 µL per well.

26. Thaw Target Probe Sets (20X) at room temperature.
27. Pre-warm PrimeFlow™ RNA Target Probe Diluent to 40°C.
28. Dilute Target Probes 1/20 in PrimeFlow™ RNA Target Probe Diluent. Mix thoroughly by pipetting up and down.

    Note: You will need 100 µL of diluted Target Probes for each sample. If you are adding more than one Target Probe per sample, adjust the volume of the PrimeFlow™ RNA Target Probe Diluent accordingly so that the final volume remains 100 µL per sample.
29. Add 100 µL of PrimeFlow™ RNA Wash Buffer to each well. Then add 100 µL of diluted Target Probe(s) directly into the cell suspension for the appropriate samples and pipet to mix. Incubate plate with the lid on for 2 hours at 40°C.

    Note: Plate sealing is not necessary for hybridization. Plates should be placed directly onto the incubator shelf. Do not stack plates.
30. Spin down at 1,000 x g for 4 minutes at room temperature. Discard supernatant and resuspend cells in the residual volume by vortexing gently.
31. Add 200 µL of PrimeFlow™ RNA Wash Buffer, and spin down at 1,000 x g for 4 minutes at room temperature. Discard supernatant and resuspend cells in the residual volume by vortexing gently.
32. Repeat Step 31.
33. Prepare PrimeFlow™ RNA Wash Buffer with RNase Inhibitors by adding RNase Inhibitors (100X) to PrimeFlow™ RNA Wash Buffer at a 1/100 dilution. Mix gently by inverting.

    Note: You will need 100 µL of this buffer per well. Prepare this buffer in bulk to accommodate all samples. This buffer should be prepared fresh. Dispose of any unused buffer.
34. Add 100 µL of PrimeFlow™ RNA Wash Buffer with RNase Inhibitors, and pipet to mix.
35. Store samples overnight in the dark at 2–8°C with the lid on.

    Note: We recommend this stopping point for ease-of-use and a more manageable workflow. However, if desired, Step 35 may be skipped. If skipping this step, proceed to Step 36 and continue through to the end of the protocol.

Day 2: Signal amplification

Note: It is critical that the residual volume after all washes does not exceed 10 µL.

Note: PrimeFlow™ RNA PreAmp Mix, PrimeFlow™ RNA Amp Mix, and diluted Label Probes should be pipetted directly into the samples and mixed well before incubating. The total volume for each hybridization is 200 µL per well. During hybridization, keep the lid on the plate. Place plates directly on the incubator shelf. Do not stack plates.

36. Pre-warm samples and PrimeFlow™ RNA Wash Buffer to room temperature.

    Note: Some precipitation in the Wash Buffer may occur. After warming to room temperature, an aliquot for the volume needed for the day may be prepared and allowed to settle or briefly centrifuged to remove precipitation. Do not repeatedly centrifuge the Wash Buffer to remove precipitation.
37. Pre-warm PrimeFlow™ RNA PreAmp Mix, PrimeFlow™ RNA Amp Mix, and PrimeFlow™ RNA Label Probe Diluent to 40°C.
38. Thaw PrimeFlow™ RNA Label Probes (100X) on ice in the dark.

    Note: This can be done during the Amp Mix incubation (Step 43).
39. Add 100 µL of PrimeFlow™ RNA PreAmp Mix directly into the cell suspension for each sample, and pipet to mix, and then incubate plate with the lid on for 1.5 hours at 40°C.
40. Spin down at 1,000 x g for 4 minutes at room temperature. Discard supernatant and resuspend cells in the residual volume by vortexing gently.
41. Add 200 µL of PrimeFlow™ RNA Wash Buffer, and spin down at 1,000 x g for 4 minutes at room temperature. Discard supernatant and resuspend cells in the residual volume by vortexing gently.
42. Repeat Step 41.

43. Add 100 µL of PrimeFlow™ RNA Wash Buffer to each well. Then add 100 µL of PrimeFlow™ RNA Amp Mix directly into the cell suspension, and pipet to mix. Incubate plate with the lid on for 1.5 hours at 40°C.
44. Spin down at 1,000 x g for 4 minutes at room temperature. Discard supernatant and resuspend cells in the residual volume by vortexing gently.
45. Add 200 µL of PrimeFlow™ RNA Wash Buffer, and spin down at 1,000 x g for 4 minutes at room temperature. Discard supernatant and resuspend cells in the residual volume by vortexing gently.
46. Repeat Step 45.
47. Dilute PrimeFlow™ RNA Label Probes (100X) 1/100 in PrimeFlow™ RNA Label Probe Diluent.

    Note: You will need 100 µL of diluted Label Probes for each sample. Prepare diluted Label Probes in bulk to accommodate all samples.
48. Add 100 µL of PrimeFlow™ RNA Wash Buffer to each well. Then add 100 µL of diluted PrimeFlow™ RNA Label Probes directly into the cell suspension, and pipet to mix. Incubate plate with the lid on for 1 hour at 40°C.
49. Spin down at 1,000 x g for 4 minutes at room temperature. Discard supernatant and resuspend cells in the residual volume by vortexing gently.
50. Add 200 µL of PrimeFlow™ RNA Wash Buffer, and spin down at 1,000 x g for 4 minutes at room temperature. Discard supernatant and resuspend cells in the residual volume by vortexing gently.
51. Repeat Step 50.
52. Add 200 µL of PrimeFlow™ RNA Storage Buffer or Flow Cytometry Staining Buffer, and spin down at 1,000 x g for 4 minutes at room temperature. Discard supernatant and resuspend cells in the residual volume by vortexing gently.
53. If a plate adapter is used for sample acquisition, resuspend cells in an appropriate volume of PrimeFlow™ RNA Storage Buffer or Flow™ Cytometry Staining Buffer, and analyze on a flow cytometer. Otherwise, transfer samples to 12 x 75 mm polystyrene tubes, resuspend cells in an appropriate volume of PrimeFlow™ RNA Storage Buffer or Flow Cytometry Staining Buffer, and analyze on a flow cytometer.

    Note: Samples may be stored in the dark at 2–8°C for up to three days before analysis. We recommend storing the samples in IC Fixation Buffer by mixing 100 µL of cells with 100 µL of IC Fixation Buffer.

Quick guide: PrimeFlow™ RNA Assay with microRNA pretreatment in 96-well plates

Day 1

1. Surface stain cells with antibody and/or fixable viability dye and prepare compensation controls.
2. Wash once with Flow Cytometry Staining Buffer. Spin cells at 500 x g for 4 minutes at 2–8°C.
3. Incubate cells in 1X PrimeFlow™ microRNA Pretreatment Buffer for 15 minutes at room temperature. Then spin cells at 1,000 x g for 4 minutes at 2–8°C.
4. Wash once with 1X PBS. Spin cells at 1,000 x g for 4 minutes at 2–8°C.
5. Fix cells in PrimeFlow™ RNA Fixation Buffer 1 for 30 minutes at 2–8°C.
6. Wash twice with 1X PrimeFlow™ RNA Permeabilization Buffer with RNase Inhibitors. Spin cells at 1,000 x g for 4 minutes at 2–8°C.
7. Intracellularly stain cells with antibody for 30 minutes at 2–8°C.
8. Wash once with 1X PrimeFlow™ RNA Permeabilization Buffer with RNase Inhibitors. Spin cells at 1,000 x g for 4 minutes at 2–8°C.
9. Fix cells in 1X PrimeFlow™ RNA Fixation Buffer 2 for 60 minutes at room temperature.
10. Wash twice with PrimeFlow™ RNA Wash Buffer. Spin cells at 1,000 x g for 4 minutes at room temperature.
11. Perform Target Probe hybridization for 2 hours at 40°C.
12. Wash twice with PrimeFlow™ RNA Wash Buffer. Spin cells at 1,000 x g for 4 minutes at room temperature.
13. Resuspend cells in PrimeFlow™ RNA Wash Buffer with RNase Inhibitors.
14. Store samples overnight.

Day 2

15. Perform PreAmp hybridization for 1.5 hours at 40°C.
16. Wash twice with PrimeFlow™ RNA Wash Buffer. Spin cells at 1,000 x g for 4 minutes at room temperature.
17. Perform Amp hybridization for 1.5 hours at 40°C.
18. Wash twice with PrimeFlow™ RNA Wash Buffer. Spin cells at 1,000 x g for 4 minutes at room temperature.
19. Perform Label Probe hybridization for 1 hour at 40°C.
20. Wash twice with PrimeFlow™ RNA Wash Buffer. Spin cells at 1,000 x g for 4 minutes at room temperature.
21. Wash once with PrimeFlow™ RNA Storage Buffer or Flow Cytometry Staining Buffer. Spin cells at 1,000 x g for 4 minutes at room temperature.

Specificity	Clone	PrimeFlow RNA Assay Standard protocol	PrimeFlow RNA Assay with microRNA Pretreatment
Human AHR	FF3399	++	++
Human EOMES	WD1928	+++	++
Human Foxp3	PCH101	++	-
Human Foxp3	236A/E7	++	-
Human/Mouse Gata-3	TWAJ	nd[a]	nd
Human IFN gamma	4S.B3	+++	+++
Human IL-17A	eBio64DEC17	+++	+++
Human IL17AF	20LJS09	+++	+++
Human IL-2	MQ1-17H2	+++	+++
Human Ki-67	20Raj1	+++	++
Human Perforin	dG9 (delta G9)	+++	++
Human/Mouse T-bet	eBio4B10 (4B10)	++	+
Human TNF alpha	MAb11	+++	+++
Mouse EOMES	Dan11mag	++	+
Mouse/Rat Foxp3	FJK-16s	++	-
Mouse Granzyme B	NGZB	+++	+++
Mouse IFN gamma	XMG1.2	+++	+++
Mouse IL-10	JES5-16E3	nd	nd
Mouse IL-13	eBio13A	++	++
Mouse/Rat IL-17A	eBio17B7	+++	+++
Mouse IL-2	JES6-5H4	+++	++
Mouse IL-4	11B11	++	++
Mouse/Rat Ki-67	SolA15	+++	++
Mouse ROR gamma (t)	B2D	nd	nd
Mouse TNF alpha	MP6-XT22	+++	+++

Table 1: Performance of antibodies for intracellular targets[b]

[a] *Testing not done.*
[b] *Antibody performance was assessed after the fixation and permeabilization steps according to the protocols indicated. Additional changes in antibody performance are expected following completion of the entire protocol.*

Contact Thermo Fisher Technical Support for more details.

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Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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