PRODUCT SHEET | DAHLIA RNA FLOW ASSAYS

Ultra-throughput single-cell RNA analysis

Measure gene expression in single cells by flow cytometry

INTRODUCTION

Dahlia RNA Flow Assays are the easiest way to measure single-cell gene expression and seamlessly integrate into existing flow cytometry workflows. Using proprietary Proximal Two-Probe hybridization chemistry and rolling circle amplification, Dahlia RNA Flow Assays enable high-throughput single-cell RNA analysis in less time than competing flow methods.

⊙ Seamless Adoption
Use with any fluorescence-based flow cytometer and get data in less than a day with minimal hands-on time.

⊙ Multi-Color Analysis
Detect multiple RNA targets with single-cell resolution.

⊙ Antibody Co-Staining
Combine with compatible antibody staining panels.

⊙ Rapid Assay Design & Delivery
Proprietary design pipeline yields fully wet-lab-validated RNA probe sets against any target sequence, delivered within 3 weeks.

Streamlined process that plugs into existing workflows

Samples can be run using existing PCR thermal cyclers and flow cytometers.

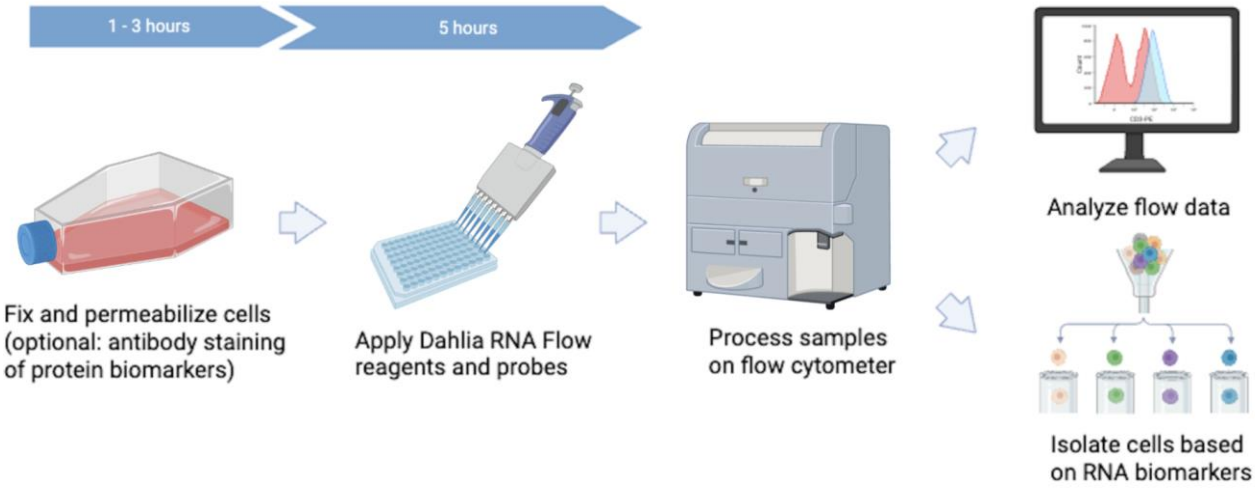


FIG 1. Dahlia RNA Flow Assay 4-step workflow. Step 1. Single-cell suspensions are fixed and permeabilized in 1.6% PFA and methanol. As an optional step, surface markers can be stained with antibodies for co-detection and cross-linked. Step 2. Dahlia RNA Flow Assays are applied to the sample. RNA detection steps can be completed in under 5 hours. Step 3. Samples are then run through flow cytometry workflow. Step 4. Further analysis or cell sorting is performed based on target RNA expression.

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Multi-Color Analysis

Dahlia RNA Flow Assays can currently detect up to 3 RNA targets in 3 spectrally distinct fluorophores (Cy5, Cy3, FITC).

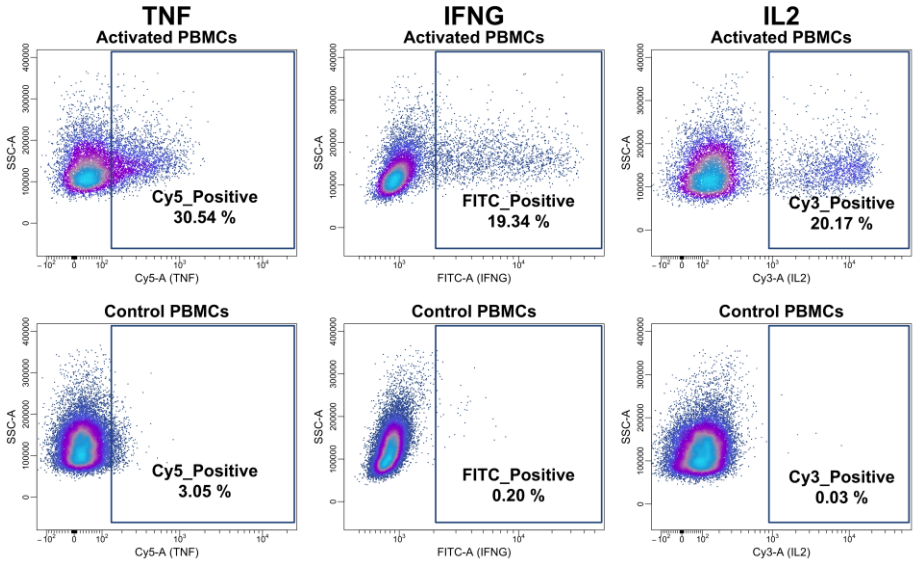


FIG.2. Multiplex detection of cytokine gene expression. Human PBMCs were stimulated with a cocktail of PMA/Ionomycin, or a control cocktail without stimulation reagents. Cells were then subjected to Dahlia's RNA Flow protocol with multiplex hybridization of three cytokine assays, TNF, IFNG, and IL2, assigned to the Cy5, FITC, and Cy3 channels respectively. Control and activated PBMC expression levels were verified by qPCR, which confirmed low baseline TNF expression in control cells.

Antibody Co-Staining

Surface marker antibody staining can be done during the sample prep step and co-detected with RNA target(s). Antibodies must be compatible with methanol-based fix and perm, and formamide.

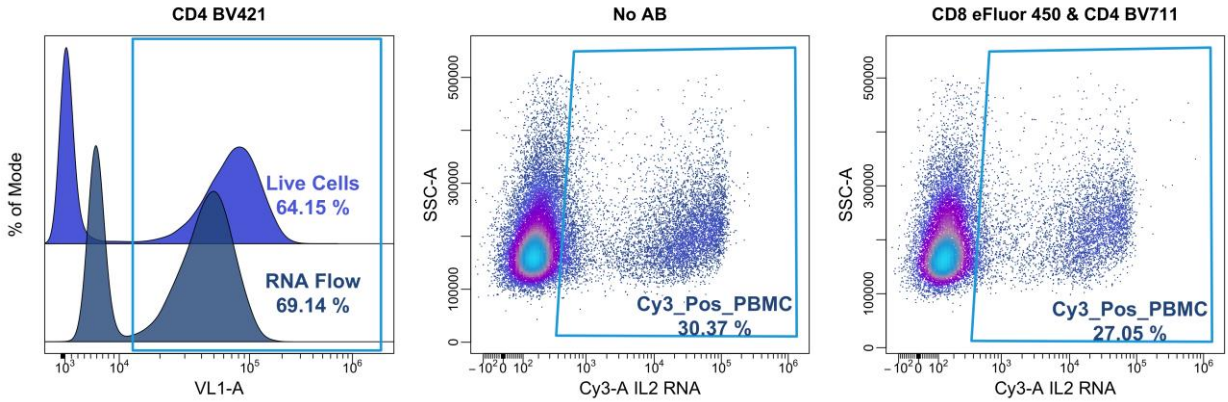


FIG.3. Activated PBMCs were first stained with various surface markers, and the antibody protein conjugates were cross-linked prior to fixation and permeabilization. These antibody surface-stained cells were then subjected to the RNA Flow protocol. Panel 1 shows the impact of fix/perm and the RNA Flow protocol on the signal of CD4 BV421. Panels 2 and 3 show the detection of IL2 RNA with and without antibody surface staining (CD8 eFluor™ 450 & CD4 BV711) prior to the RNA Flow protocol. In both cases, there is minimal impact.

Rapid Assay Design & Delivery

Dahlia RNA Flow Assays can be created for any target sequence down to 50 nt, including splice variants. This allows for the detection of biomarkers that may not have functional antibodies. All assays are wet-lab-validated and delivered within 3 weeks.

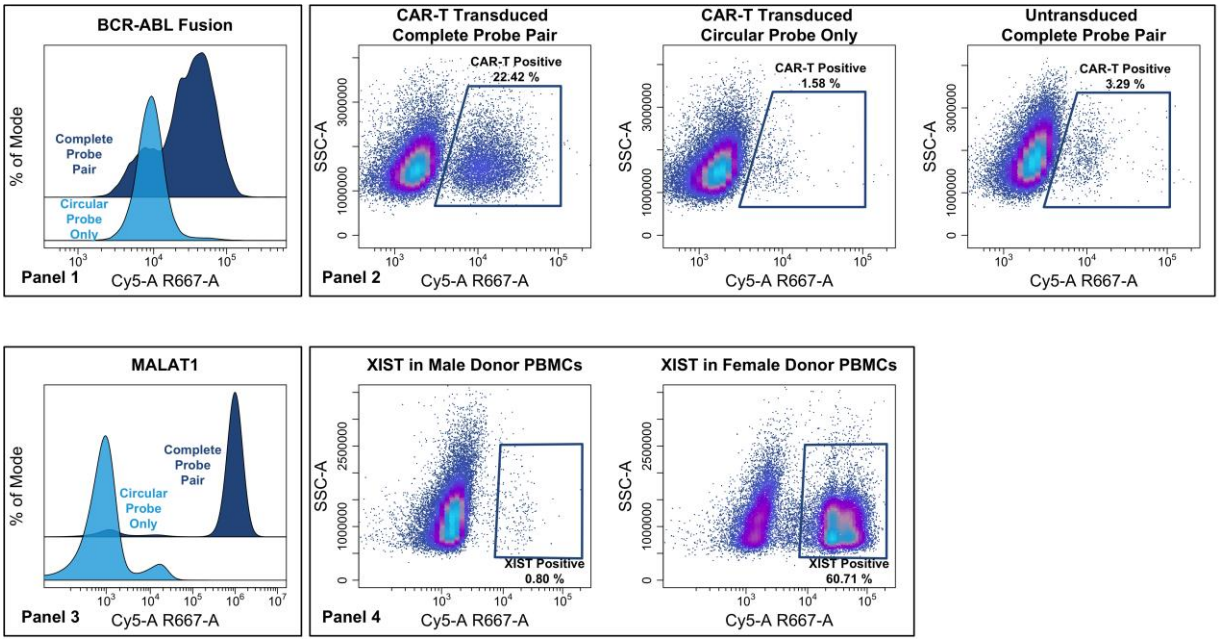


FIG.4. Panel 1: Detection of BCR-ABL fusion gene in K562 cell line. Panel 2: Detection of CAR-T gene in transduced cells. Panel 3: Detection of lncRNA MALAT1 in human PBMCs. Panel 4: Detection of lncRNA XIST in female donor PBMCs. XIST positivity in female donor PBMCs is consistent with published scRNA-seq data, in which XIST is detected in about half of female T cells (calculated from the Allen Institute Human Immune Health Atlas).

PRODUCT ORDERING INFORMATION

PRODUCT	DESCRIPTION	PART NUMBER
Dahlia RNA Flow Assay Kit (small, large)	Includes cell resuspension buffer, probe hybridization mix, amplification mix and enzymes and wash buffers for 25 or 100 reactions	DB-RFK-01-SM DB-RFK-01-LG
Dahlia RNA Flow Probe Set (small, large)	Target-specific probes and fluorescent detection oligos (Cy5, Cy3, FITC) for 25 or 100 reactions	DB-RFA-XXXX-SM DB-RFA-XXXX-LG XXXX = target ID assigned at order

Contact us!

Submit your RNA targets of interest or talk to our scientists.

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