

# TruCulture®-NanoString® Workflow

## Sample Extraction and Preparation

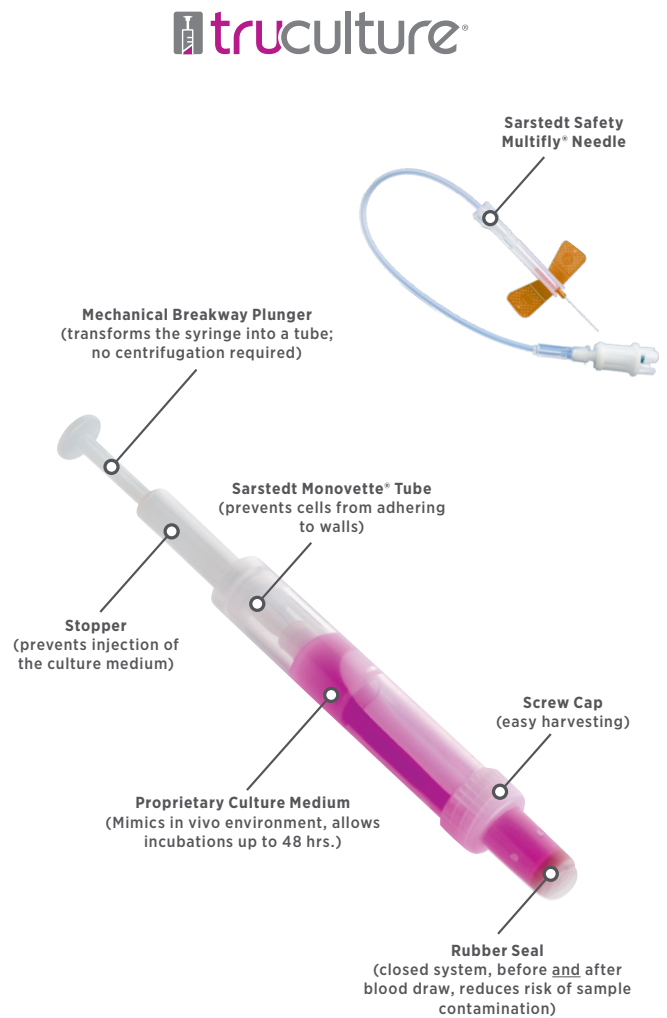
1. After incubation, separate cells from supernatant using Seraplas valve and plunger
2. Remove supernatant
3. Remove Seraplas valve
4. Remove cell layer (~1mL) using a pipette to fresh 15 mL conical tube containing 3 mL RNAlater
5. Once resuspended in RNAlater, cells can be stored or immediately processed by RNA extraction

## RNA Extractions

6. Gently vortex sample tube for 5 seconds to mix cells
7. Remove appropriate volume of cell suspension for RNA extraction method being used
  - RNA is extracted using the Ambion RiboPure RNA kit with a recommended column capacity of 500  $\mu$ L whole blood
  - 2 mL cell suspension (cell equivalent of 500  $\mu$ L whole blood) is aliquoted to a fresh tube for the Ambion protocol
8. Add 2 mL PBS and mix thoroughly
9. Centrifuge at 2000g for 10 minutes (until cells are pelleted) and remove supernatant
10. Continue with step B1.b of the Ambion RiboPure protocol, and continue with protocol to completion

*Note: The Ambion protocol recommends 100  $\mu$ L elution volume. RNA concentration may be increased by reducing elution volume if desired*

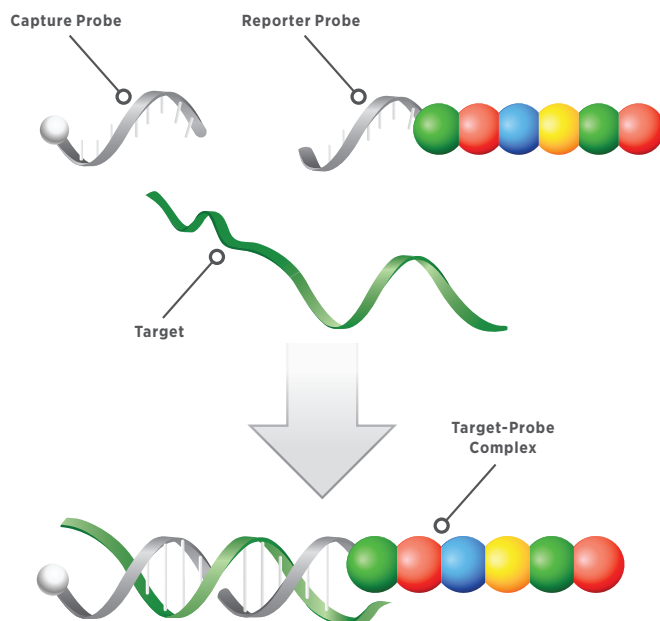
(cont.)



## TruCulture®-NanoString® Workflow (cont)

### RNA QC and NanoString Assays

11. Measure RNA concentration by A260 UV absorbance (e.g., Nanodrop) or alternatively by fluorescent dye method (e.g., Qubit)
  - Normalize RNA to a single concentration (e.g., 20 ng/μL for a 100ng total concentration) by dilution in appropriate volume of molecular biology grade water.
12. Set up nCounter® hybridizations following NanoString MAN-10023 and continue with downstream processing and data collection as appropriate for your nCounter system.
  - Recommended minimum RNA input is 25 ng.



For more information, visit [nanosttring.com/truculture](https://nanosttring.com/truculture)

#### NanoString Technologies, Inc.

530 Fairview Avenue North  
Seattle, Washington 98109

T (888) 358-6266  
F (206) 378-6288

nanosttring.com  
info@nanosttring.com

#### Sales Contacts

United States [us.sales@nanosttring.com](mailto:us.sales@nanosttring.com)  
EMEA: [europa.sales@nanosttring.com](mailto:europa.sales@nanosttring.com)

Asia Pacific & Japan [apac.sales@nanosttring.com](mailto:apac.sales@nanosttring.com)  
Other Regions [info@nanosttring.com](mailto:info@nanosttring.com)

FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.

NanoString, NanoString Technologies, the NanoString logo, and nCounter are trademarks or registered trademarks of NanoString Technologies, Inc., in the United States and/or other countries. All other trademarks and/or service marks not owned by NanoString that appear in this document are the property of their respective owners.