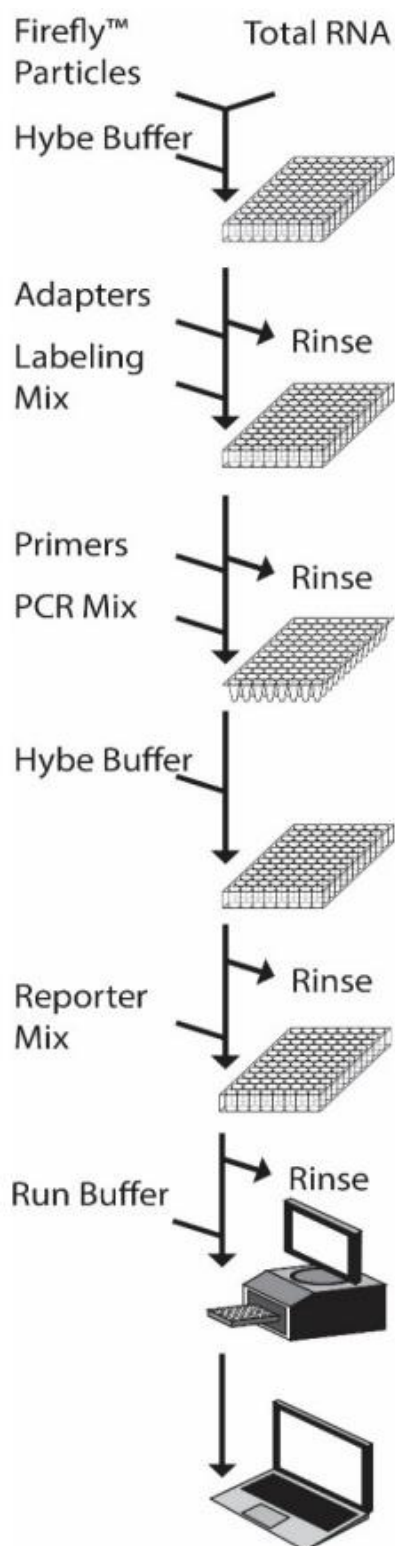


Multiplex miRNA Circulating (purified RNA) Assay workflow



Hybe:

- Add 35 μL Firefly™ particles (then apply vacuum).
- 25 μL hybe Buffer and 25 μL Total RNA.
- Shake at 37°C for 60 min. Rinse twice with 175 μL 1X rinse A.

Labeling:

- Add 75 μL 1X labeling mix with enzyme.
- Shake at room temperature for 60 min.
- Rinse twice with 165 μL 1X rinse B.
- Rinse once with 175 μL 1X rinse A.

PCR:

- Elute with 110 μL RNase-free water.
- Add 75 μL 1X rinse A to each well to store.
- Combine 30 μL eluent + 20 μL PCR master mix.
- PCR amplify.

Capture:

- Apply vacuum to clear rinse A from particles.
- Combine 20 μL PCR product + 60 μL hybe buffer.
- Shake at 37°C for 30 min.
- Rinse twice with 175 μL 1X rinse A.

Report:

- Add 75 μL 1X reporter mix.
- Shake at room temperature for 15 min.
- Rinse twice with 175 μL 1X rinse A.

Scan:

- Add 175 μL run buffer. Scan on appropriate flow cytometer.

Analyze:

- Flow cytometer files analyzed with the Firefly™ Analysis Workbench software.