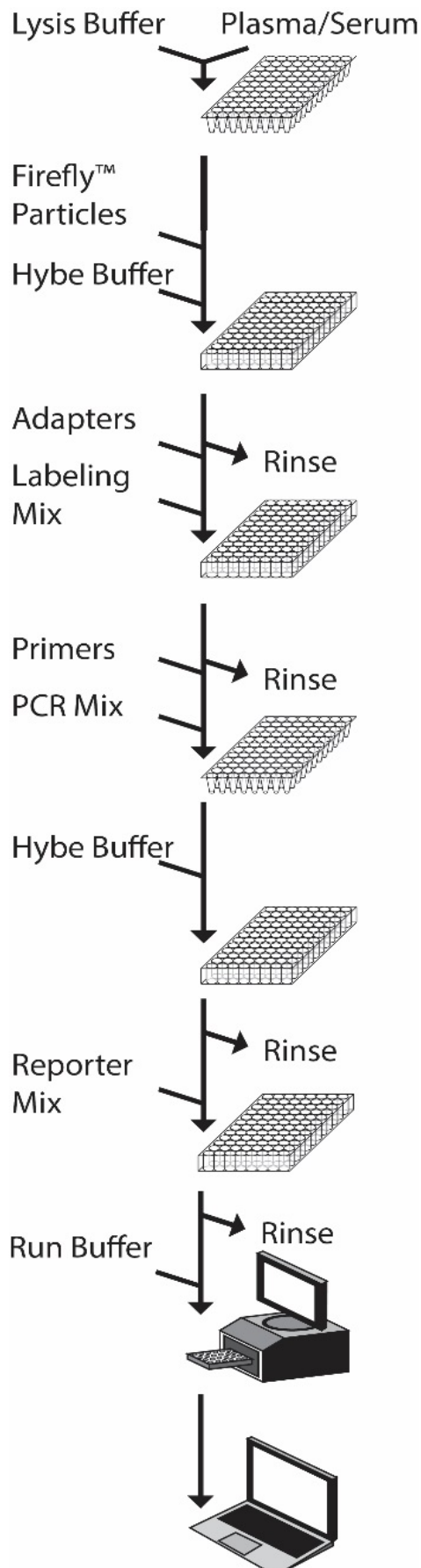


# Multiplex miRNA Circulating (crude biofluid)

## Assay workflow



**Lysis:** Combine 40  $\mu\text{L}$  sample + 40  $\mu\text{L}$  lysis buffer.

Shake at 60°C for 45 min.

**Hybe:** Add 35  $\mu\text{L}$  Firefly™ particles (then apply vacuum).

25  $\mu\text{L}$  hybe Buffer and 25  $\mu\text{L}$  digested sample.

Shake at 37°C for 60 min.

Rinse twice with 175  $\mu\text{L}$  1X rinse A.

**Labeling:** Add 75  $\mu\text{L}$  1X labeling mix with enzyme.

Shake at room temperature for 60 min.

Rinse twice with 165  $\mu\text{L}$  1X rinse B.

Rinse twice with 175  $\mu\text{L}$  1X rinse A.

**PCR:** Elute with 110  $\mu\text{L}$  RNase-free water.

Add 75  $\mu\text{L}$  1X rinse A to each well to store.

Combine 30  $\mu\text{L}$  eluent + 20  $\mu\text{L}$  PCR master mix.

PCR amplify.

**Capture:** Apply vacuum to clear rinse A from particles.

Combine 20  $\mu\text{L}$  PCR product + 60  $\mu\text{L}$  hybe buffer.

Shake at 37°C for 30 min.

Rinse twice with 175  $\mu\text{L}$  1X rinse A.

**Report:** Add 75  $\mu\text{L}$  1X reporter mix.

Shake at room temperature for 15 min.

Rinse twice with 175  $\mu\text{L}$  1X rinse A.

**Scan:** Add 175  $\mu\text{L}$  run buffer. Scan on appropriate flow cytometer.

**Analyze:** Flow cytometer files analyzed with the Firefly™ Analysis Workbench software.