Supplementary Information

Encoded hydrogel microparticles for sensitive and multiplex microRNA detection directly from raw cell lysates

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Table S1. Probe Pre-polymer compositions. The above solutions were diluted 9:1 in 1 mg/mL acryl-rhodamine (code region), 1X TE (spacer region), or DNA probe solution (probe region) prior to placement in the microfluidic channel for stop flow lithography. PEG-DA and PEG 200 were purchased from Sigma-Aldrich. 100X TE was purchased from EMD Millipore and diluted with Nuclease Free Water (Affymetrix). Probes were purchased lyophilized from IDT Technologies and resuspended in 1X TE buffer.

	Code, Spacer	Probe
Polyethylene Glycol Diacrylate (PEG-DA-700)	35%	20%
Polyethylene Glycol (PEG 200)	20%	40%
Darocur	5%	5%
3X Tris-EDTA Buffer (3X TE)	40%	35%

Table S2. Nucleic acid sequences.

miR-21 probe	5'-/5Acryd/GAT ATA TTT TAT CAA CAT CAG TCT GAT AAG CTA/3InvdT/-3'
miR-145 probe	5'-/5Acryd/ GAT ATA TTT TAA GGG ATT CCT GGG AAA ACT GGA C/3InvdT/-3'
miR-146a probe	5'-/5Acryd/GAT ATA TTT TAA ACC CAT GGA ATT CAG TTC TCA/3InvdT/-3'
cel-miR-54 probe	5'-/5Acryd/GAT ATA TTT TAC TCG GAT TAT GAA GAT TAC GGG TA/3InvdT/-3'
miR-21 target	5'-UAG CUU AUC AGA CUG AUG UUG A-3'
Biotinylated linker	5'-/5Phos/TAA AAT ATA TAA AAA AAA AAA A/3Bio/-3'

Table S3. Ligation buffer compositions. The buffer for the ligation portion of the labeling steps consisted of the elements in the table added to 1X TE buffer.

Buffer component	Amount
Tween-20	0.05% (v/v)
NaCl	8.8 mM
NEBuffer 2 (New England Biolabs)	8% (v/v)
ATP (New England Biolabs)	200 μM
A12 universal linker (New England Biolabs)	33 nM
T4 DNA ligase	658 U/mL

Supporting Figures

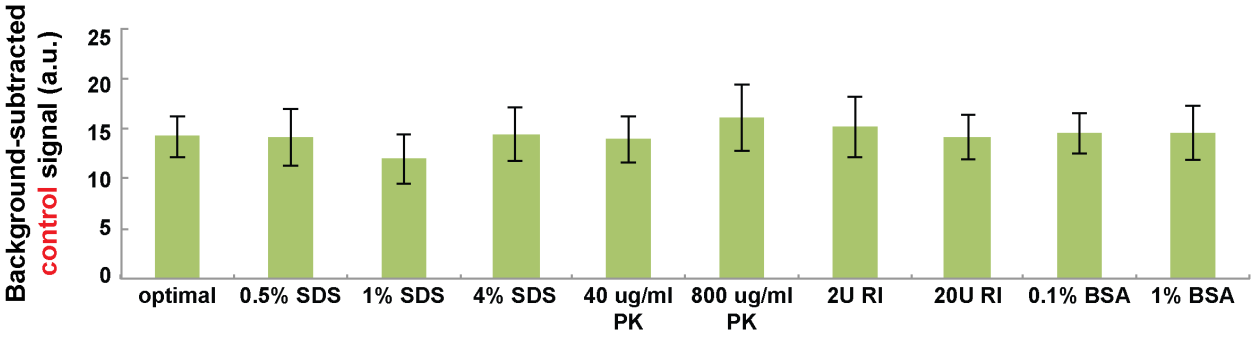

Figure S1. Comparison of background-subtracted control signal for various buffer conditions. The background-subtracted control signal remained roughly constant with varying concentrations of dodecyl sodium sulfate (SDS), Proteinase K (PK), RNase inhibitor (RI), and bovine serum albumin (BSA). SDS and BSA concentrations are in % (w/v) and RI amounts are in Units. Error bars represent standard deviation (n=4-11).

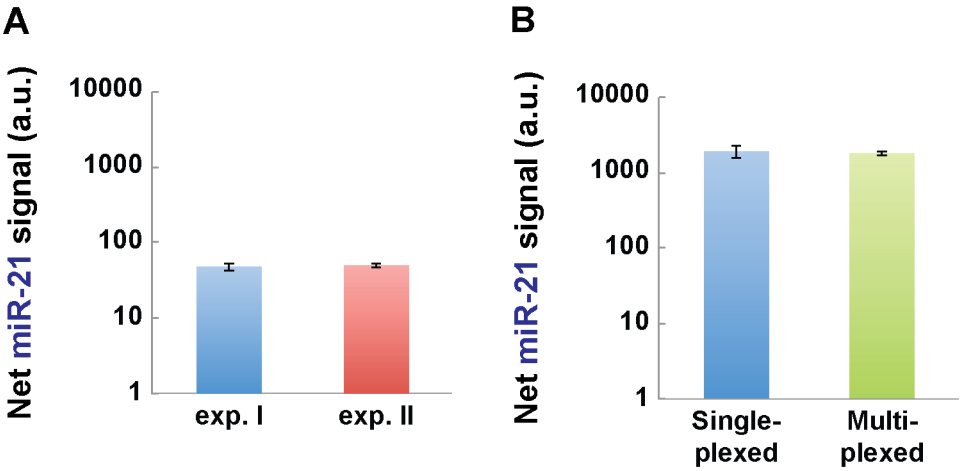

Figure S2. Fluorescent signals were similar from trial to trial. (A) Fluorescent signal comparison for two experiments on two different days with 5,000 3T3 cells. Net signals from the two experiments were roughly constant. Error bars represent standard deviation (n=4-5). (B) Similar fluorescent signals from single and multiplex assays on different days with 250,000 3T3 cells. Error bars represent standard deviation (n=8-9).

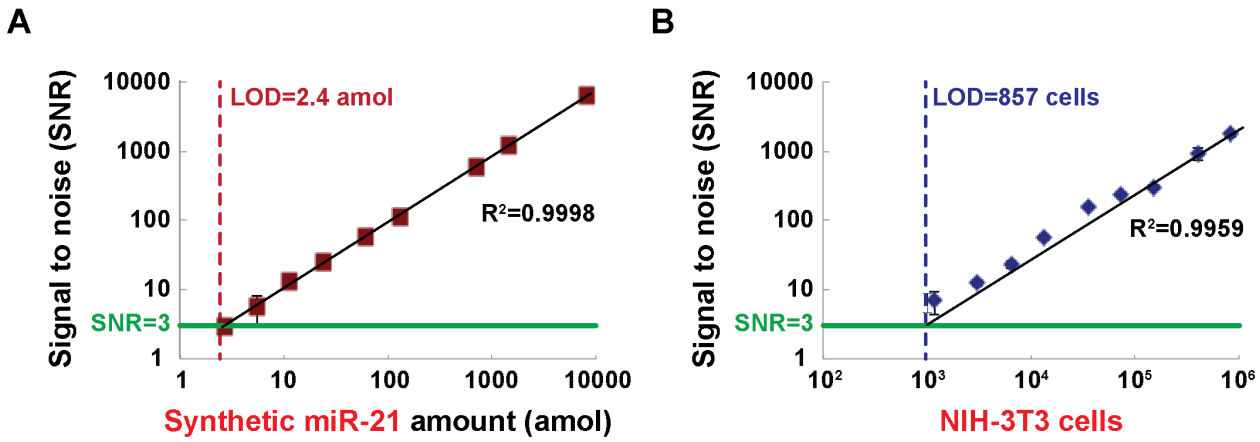

Figure S3. Determination of the limit of detection (LOD) of the assay. To calculate the limit of detection, the miR-21 signal to noise ratio (SNR) was plotted (A) as a function of the amount of synthetic miRNA-21 or (B) as a function of the number of cells added to the assay. A line was fit to the data and extrapolated to find the amount of miR-21/number of cells at which SNR=3. This point was defined to be the LOD of the assay. The calibration curves were very linear with $R^2 > 0.99$ and the LOD was ~2.4 amol synthetic miR-21 or ~857 cells. Error bars represent standard deviation (n=4-8).

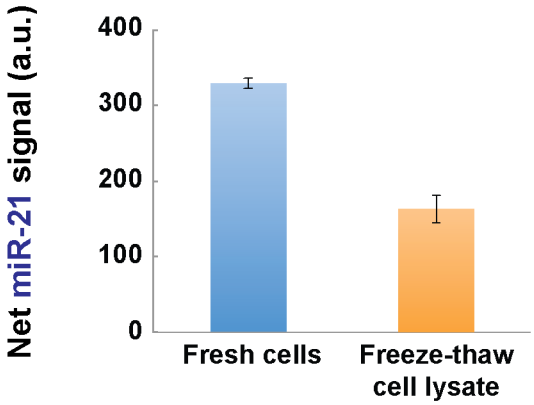

Figure S4. Freeze-thaw cell lysate exhibited ~50% lower fluorescence than fresh cell lysate. Cells were lysed in lysis buffer prior to freezing at -20 °C. Subsequent assay steps remained the same. Error bars represent standard deviation (n=5-7).