Supporting Information

Frequency-Enhanced Transferrin Receptor Antibody-Labelled Microfluidic Chip (FETAL-Chip) Enables Efficient Enrichment of Circulating Nucleated Red Blood Cells for Non-invasive Prenatal

Diagnosis

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Device fabrication and surface modification

(1) Introduction of SU-8 micro-mold onto a Si wafer

A soft lithography technique was used to fabricate the micromold. The silica wafers were placed in boiling Piranha solution $(H_2SO_4/H_2O_2, 4:1 v/v)$ for one hour and then rinsed several times with deionized water. The clean silica wafers were spin-coated with SU-8 2015 and soft baked for 5 min at 95 °C. The FETAL-Chip pattern on a film photomask was transferred onto the photoresist via UV exposure for 20s. After postexposure baking and washing away the unexposed photoresist, the SU-8 mold was successfully made on the Si wafter, showed in Fig. S1-A.

(2) Fabrication of PDMS replicates

PDMS base and curing agent were prepared in 10:1 (w/w) ratio and then mixed well and degassed. The pre-PDMS mixture was poured onto the silica wafer and spin-coated for several minutes. A vacuum desiccator was used to help PDMS pre-polymer to fill into the mold for 10 min. After being thermal cured on a hot plate at 90 °C for 15 min, the PDMS replicate was ready for FETAL-Chip. The full chip was assembled by stacking a glass slide, PDMS micropillars and a 0.5 cm-thin PDMS block layer-by-layer with oxygen plasma activation, as shown in Fig. S1-B.



Fig. S1 Workflow of PDMS-based FETAL-Chip Fabrication.

Optimization of antibody concentration

Different concentrations of biotinylated antibody were injected into the streptavidin coated chip. After one hour incubation and PBS washing, anti-mouse IgG (PE) was injected and incubated for 30 min. The fluorescence signal was recorded by microscope and quantified by ImageJ. Fig. S2 shows that 20 μ g mL⁻¹ was the best concentration. All experiments followed this antibody modification.



Fig. S2 Optimization of antibody concentration.

CD 71 expression level evaluation by flow cytometry

Binding affinity of anti-CD 71 with K562 and Ramos cell lines was tested by flow cytometry. A 1 x 10^5 cell suspension was mixed with 5 µg mL⁻¹ of antibody for 30 min, followed by anti-mouse IgG (PE) staining. 10,000 cells were recorded by BD FACS. Both cells were found to be CD 71 positive and suitable for use as modelling cells.



Fig. S3 Binding affinity of Anti-CD 71 with K562 and Ramos cell lines.

Taqman Assay

qPCR mixture was prepared with the following protocol: 2 × Takara Premix Ex Taq (Probe qPCR) Master Mix, 300 nM of each amplification primer and 100 nM of the corresponding TaqMan probe in the presence of targeted DNA. The PCR program involved a 3 min initial denaturation step at 94 °C and 10 cycles pre-amplification at 94 °C for 15 s and 60 °C for 30 s, followed by 30 cycles at 94 °C for 15 s and 60 °C for 1 min with fluorescence recorded by a Step-One[™] real-time PCR (Applied Biosystems®, Massachusetts, USA).

Types	Sequences
SRY-forward primer	5'-TGG CGA TTA AGT CAA ATT CGC-3'
SRY-reverse primer	5'-CCC CCT AGT ACC CTG ACA ATG TAT T-3'
SRY Taqman	5'(FAM) AGC AGT AGA GCA GTC AGG GAG GCA GA (TAMRA)-3'
β-Globin-forward primer	5'-GTG CAC CTG ACT CCT GAG GAG A-3'
β-Globin-reverse primer	5'-CCT TGA TAC CAA CCT GCC CAG-3'
β-Globin Taqman	5'-(FAM)AAG GTG AAC GTG GAT GAA GTT GGT GG(TAMRA)-3'

Globin gene was used as an internal reference to test the quality of extracted DNA.



Fig. S4. Globin gene detection of DNA samples extracted from captured cell chip