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Supporting Information

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Efficient Capture and Simple Quantification of Circulating Tumor Cells Using Quantum Dots and Magnetic Beads

*Hyegeun Min, Seong-Min Jo, and Hak-Sung Kim**

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Materials and Methods

Modification of antibodies with thiol group

Antibody of 1 mg was reacted with SATA of 6.7 micrograms under HEPES buffer of 0.45 mL (10 mM, pH 7.4) for 45 min at room temperature (molar ratio of antibody to SATA is 1:18). After first reaction, hydroxylamine hydrochloride solution (0.5 M) of 0.05 mL was added to reactant and further incubated for 2 h. Byproduct and un-reacted reagents were removed by dextran desalting column. Concentration of antibody was determined by a Bradford assay.

Preparation of anti-EpCAM antibody-conjugated magnetic nanoparticles (anti-EpCAM -MNPs)

To fabricate maleimide-modified MNPs, oleic acid-stabilized MNP of 1 mg was mixed with distearoylphosphoethanolamine-methoxy polyethylene $glycol_{2000}$ (DSPE-PEG_{2000}) of 40 mg and distearoylphosphoethanolamine-methoxy polyethylene $glycol_{3400}$-maleimide (DSPE-PEG_{3400}-maleimide) of 4 mg in chloroform so that the total contents of maleimide is 10 %. The solvent was evaporated under vacuum. The lipid and MNP film was hydrated by 0.75 mL of deionized water (18 MΩ), then the crude mixture was homogenized by a bath type sonicator (Branson 8510, USA) for 5 min. The resultant MNP solution was passed through a PD-10 desalting column. To fabricate anti-EpCAM-MNPs, thiolated anti-EpCAM antibody of 1.2 mg and the maleimide-introduced MNPs of 1 mg was reacted under HEPES buffer (10 mM, pH 7.2) for 24 h at 4℃. Unreacted antibody was removed by a Vivaspin 300K spin filter

(Sartorius, Germany). The concentration of MNPs was determined by measuring optical density at 400 nm wavelength.

Preparation of anti-EpCAM antibody-conjugated QD630 (anti-EpCAM-QDs)

To fabricate amine-introduced QD630, QD630 of 1 mg was mixed with DSPE-PEG_{2000} of 2.5 mg and distearoylphosphoethanolamine-methoxy polyethylene $glycol_{2000}$-amine (DSPE-PEG_{2000}-amine) of 2.5 mg in chloroform so that the total contents of amine group is 50 %. The solvent was evaporated under vacuum. The lipid and MNP film was hydrated by 0.75 mL of deionized water (18 MΩ), then the mixture was homogenized by a bath type sonicator (Branson 8510, USA) for 5 min. The resulting QD solution was passed through a PD-10 desalting column. To fabricate anti-EpCAM-QDs, the amine-introduced MNPs of 0.6 mg were reacted with sulfosuccinimidyl-4-*N*-maleimidomethyl cyclohexane-1-carboxylate (sulfo-SMCC) of 0.12 mg under HEPES buffer (10 mM, pH 7.2) for 45 min at room temperature. To remove free sulfo-SMCC, the reactant was passed through a dextran desalting column. Amine-modified QD630 of 0.5 mg was incubated with thiolated antibody of 1 mg for 24 h at 4℃. Unreacted antibody was removed by a Vivaspin 300K spin filter (Sartorius, Germany). The concentration of QD was determined by measuring fluorescence intensity at 630 nm with excitation at 350 nm.

Preparation of anti-mouse IgG-conjugated magnetic beads (anti-IgG-MBs)

Anti-mouse IgG was biotinylated by biotin-PEG_4-succinimidyl (NHS-PEG_4-biotin). Anti-mouse IgG of 0.2 mg was reacted with NHS-PEG_4-biotin for 2 h at room temperature so that the molar ratio of anti-mouse IgG to the linker is 1:8. The resulting antibodies were purified by a dextran desalting column. To fabricate anti-IgG-MBs, streptavidin-MBs of 5 mg were incubated with biotinylated-IgG of 0.2 mg for 4 h at room temperature. Free antibodies were removed by magnetic isolation.

Preparation of anti-EpCAM antibody-conjugated magnetic beads (anti-EpCAM-MBs)

Anti-EpCAM antibody was biotinylated by biotin-PEG_4-succinimidyl (NHS-PEG_4-biotin). Anti-EpCAM antibody of 0.2 mg was reacted with NHS-PEG_4-biotin for 2 h at room temperature so that the molar ratio of Anti-EpCAM antibody to the linker is 1:8. The resulting antibodies were purified by a dextran desalting column. To fabricate anti-EpCAM-MBs, streptavidin-MBs of 5 mg were incubated with biotinylated-anti-EpCAM antibody of 0.2 mg for 4 h at room temperature. Free antibodies were removed by magnetic isolation.

Cell culture and Animals

SK-Br3 (human breast carcinoma cell line), H460 (human lung adenocarcinomas) and PC9 (human lung adenocarcinomas) were kindly donated by Samsung Medical Center (Seoul, Korea). HL-60 (human promyelocytic leukemia cells) and Jurkat E6-3 (human leukemia cells) were obtained from Korean Cell Line Bank (Seoul, Korea). All cell lines were maintained in RPMI1640 media (Invitrogen 11875) supplemented with 10 % fetal bovine serum (FBS, Invitrogen 16000) without any antibiotics. All cells were cultured in a 5% CO_2 incubator at 37^{o}C (MCO-5AC; Sanyo, Japan). BALB/c female mice (6 weeks) were obtained from Orient Bio (Sungnam, Korea), and they were fed for 15 weeks.

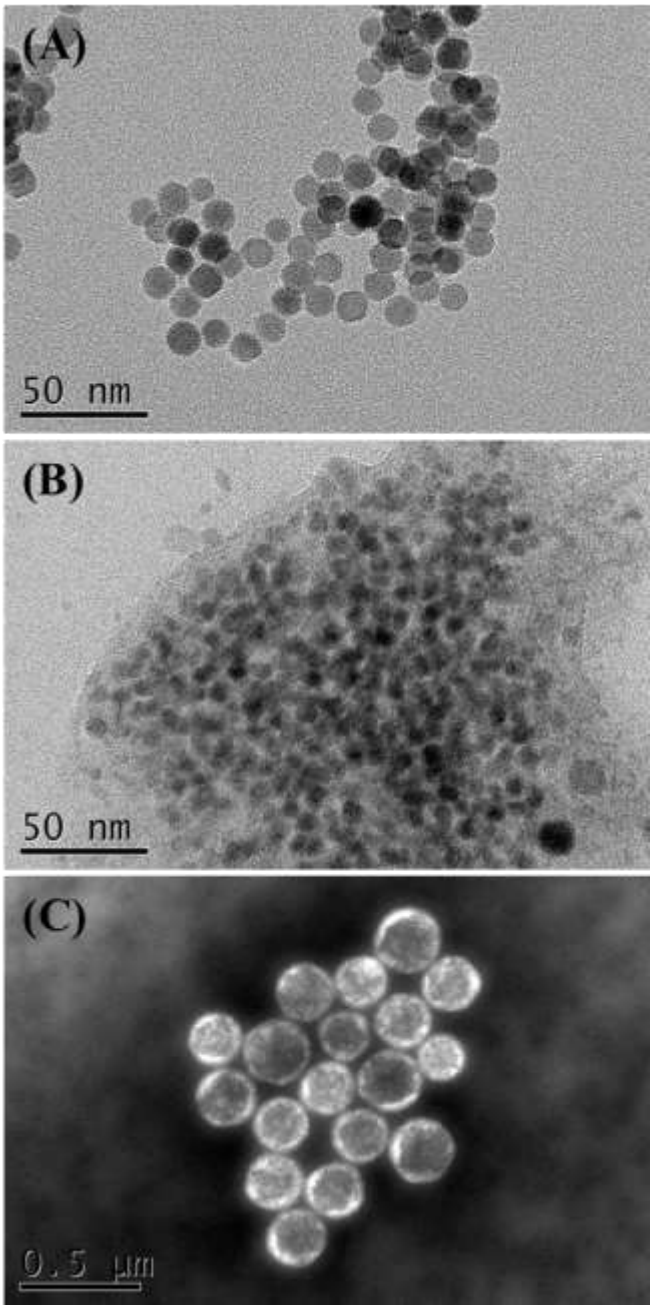

Figure S1. TEM micrographs of anti-EpCAM-MNPs(15 nm in diameter) **(A)**, anti-EpCAM-QDs **(B)**, and anti-IgG-MBs **(C)**. Magnification is 100,000-fold, 100,000-fold, and 10,000-fold, respectively. All replicas were stained by 2% phosphotungstic acid solution.

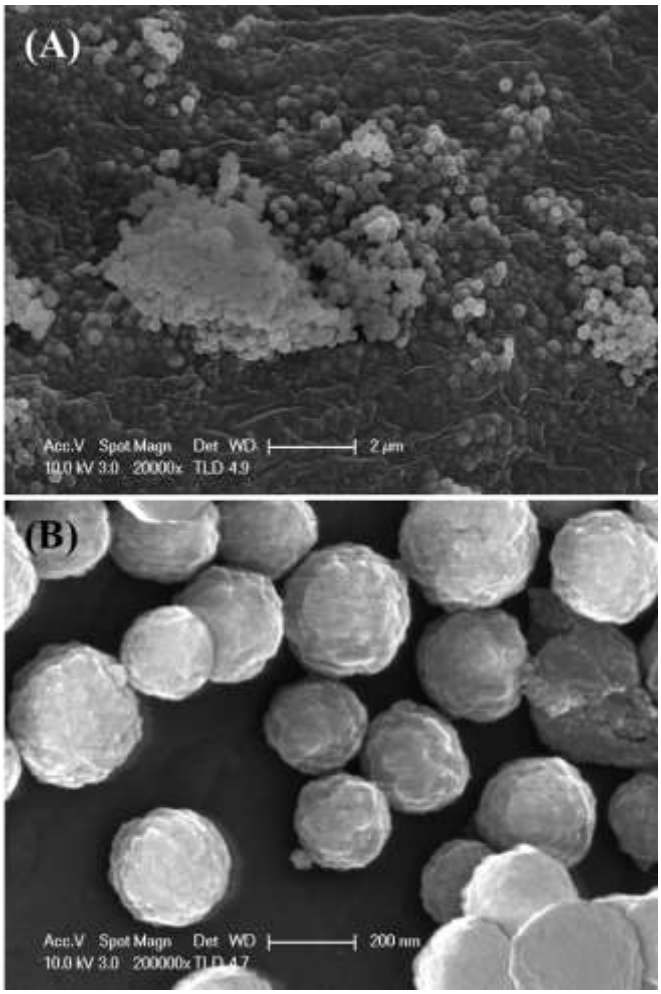

Figure S2. SEM images of anti-IgG-MBs. Conditions are 30 mA, and 120 s Pt sputtering, and approximately 10 nm coating thickness. Magnification is 2000-fold **(A)**, and 200,000-fold **(B)**, respectively.

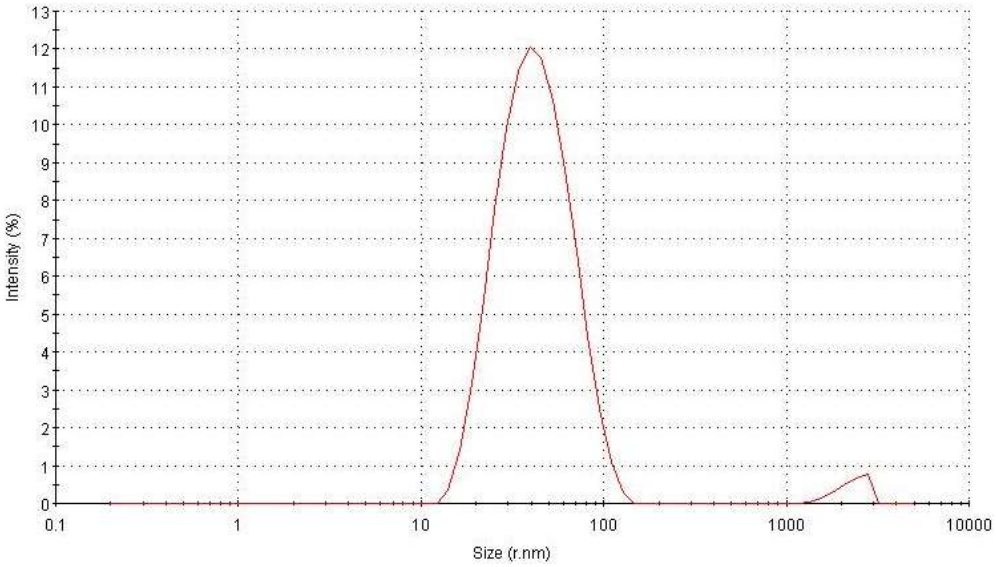

Figure S3. Hydrodynamic diameter of Anti-EpCAM-QDs by DLS (Zetasizer Nano ZS, Malvern Instruments, UK).

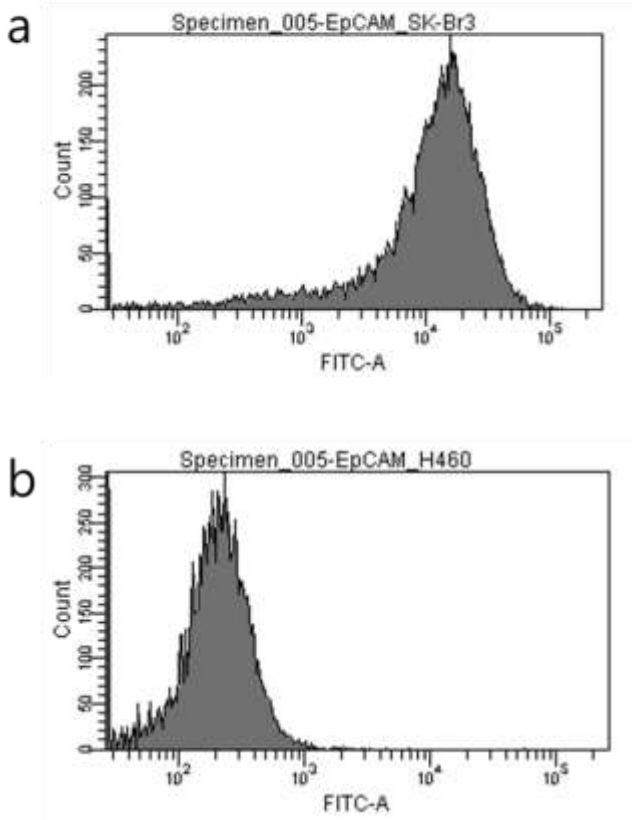

Figure S4. FACS analysis of cancer cells expressing different levels of EpCAM. The cells were incubated with FITC-labeled anti-EpCAM antibody followed by a washing, and were subjected to analysis. (a) SK-Br3. (b) H460.

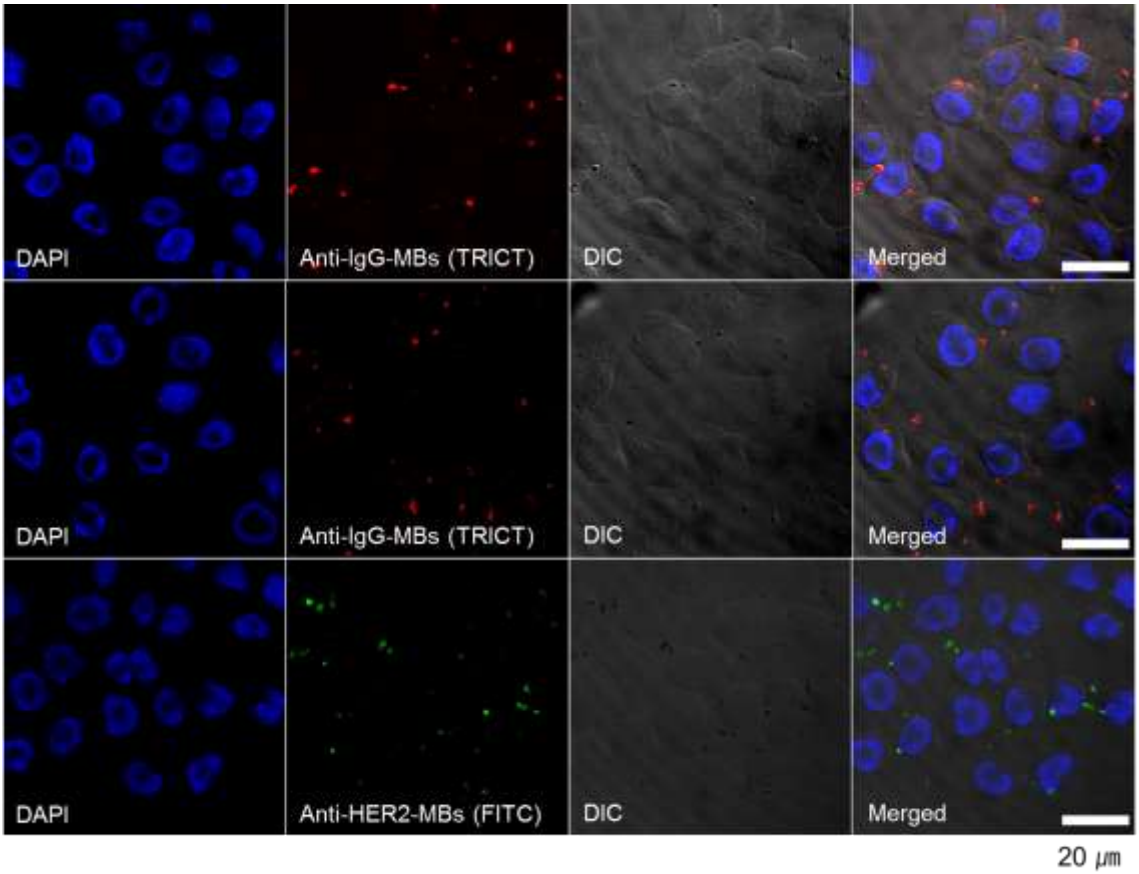

Figure S5. Confocal laser microscope images of H460 cells, treatment of anti-EpCAM-QDs and anti-IgG-MBs (top), treatment of only anti-IgG-MBs (center), and treatment of only anti-HER2-MBs (bottom). Red signals indicate the anti-IgG-MBs stained by anti-goat IgG-TRICT, and green signals indicate the anti-HER2-MBs stained by anti-mouse IgG-FITC. Mganification is 630-fold.

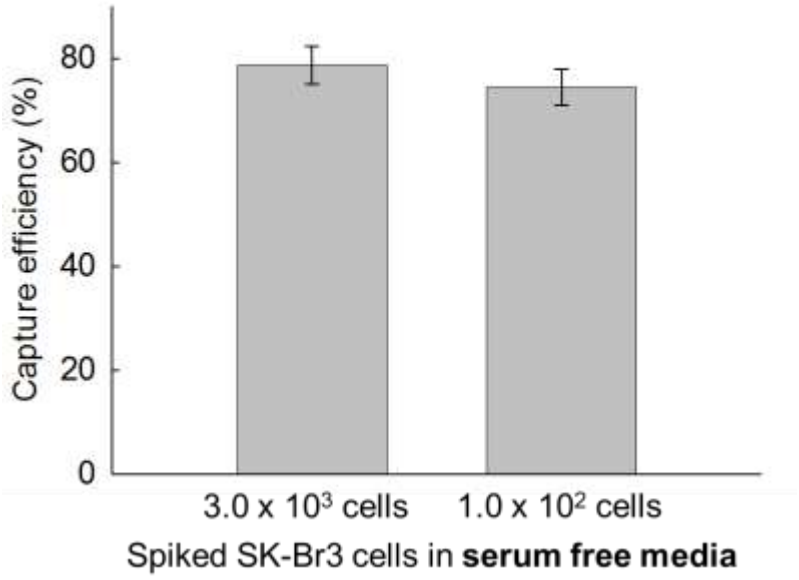

Figure S6. The capture efficiency of SK-Br3 using anti-EpCAM-QDs and anti-IgG-MBs from a serum free medium spiked with different numbers of SK-Br3 cells: 3.0×10^3 cells (left) and 1.0×10^2 cells (right).

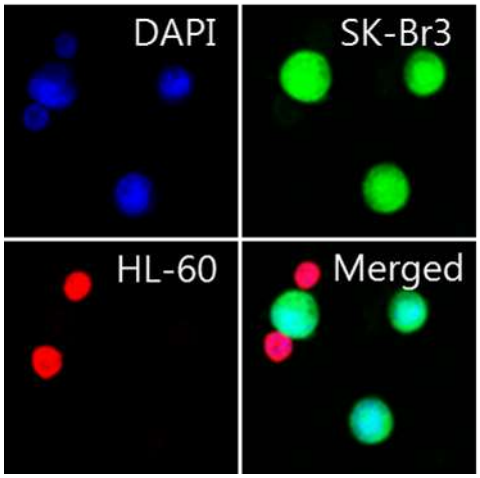

Figure S7. Confocal microscopic (mag. x400) images of cell tracker dye-stained cells after isolation

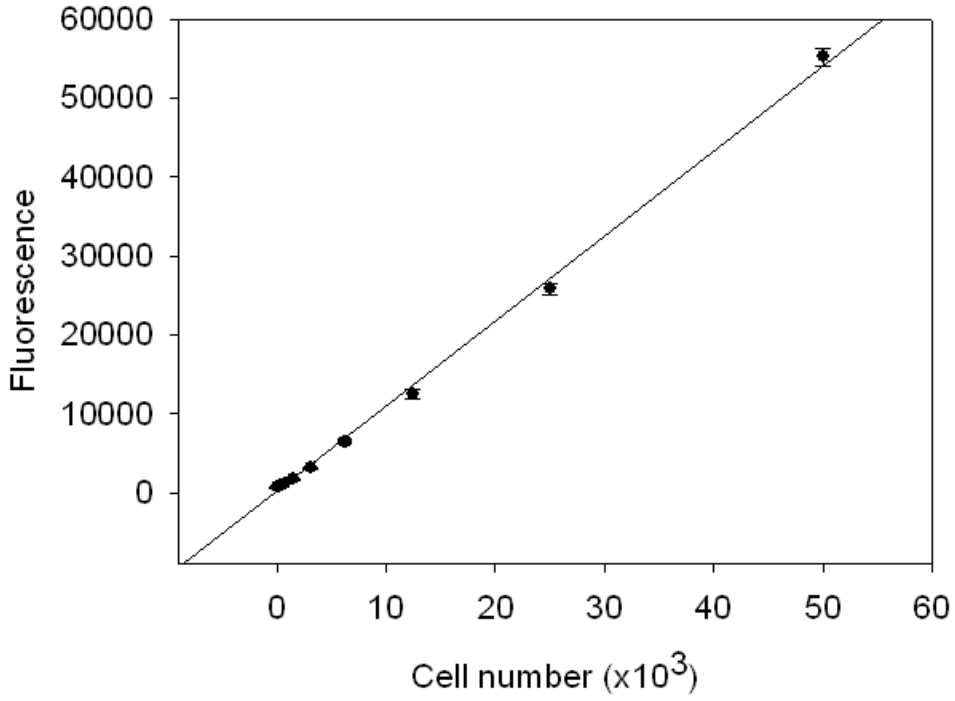

Figure S8. Calibration plot between the number of SK-Br3 cells and the fluorescence intensity from anti-EpCAM-QDs. The number of cells ranged from 1.0 x 10^1 to 5 x 10^4 in 100 microliter. .

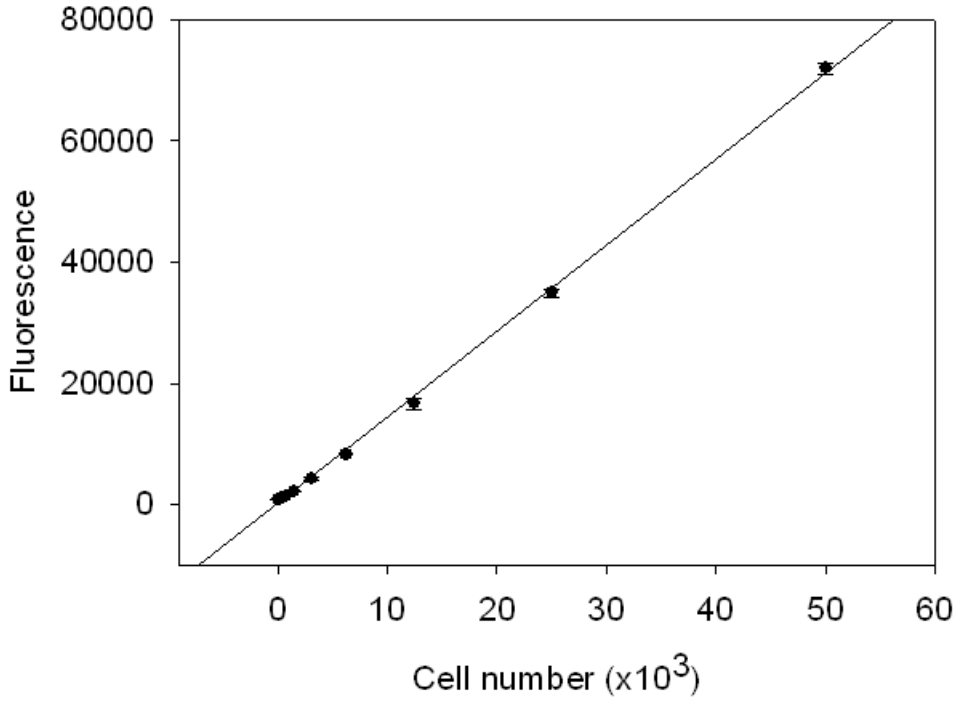

Figure S9. Calibration plot between the number of PC9 (EpCAM positive, lung adenocarcinoma cells) and the fluorescence intensity from anti-EpCAM-QDs. The number cells ranged from 1.0 x 10^1 to 5 x 10^4 in 100 microliter. .