Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

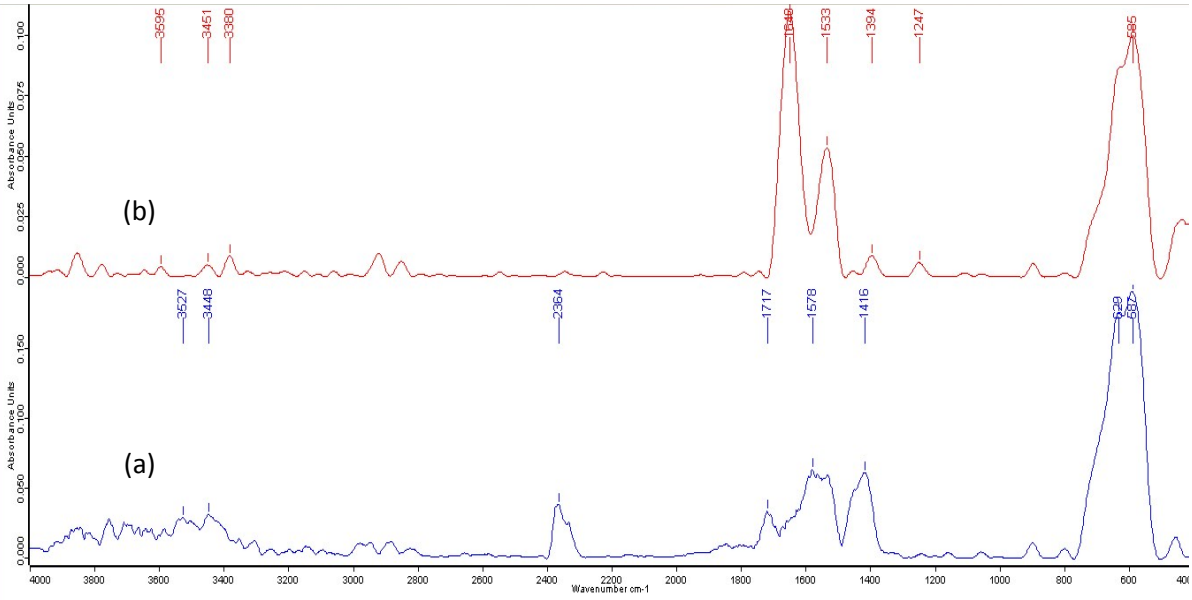


Fig. S1. FTIR spectra of functionalized MNPs. COOH-MNPs(a), Ab-MNPs (b).

The IR spectra of COOH MNPs and Ab MNPs were presented in Figure S1. A prominent absorption band at 1717 cm-1 in COOH group (Fig S1A) which was due to C=O stretch of carboxylic group. On the other hand, in Ab MNPs (Figure S1B), bands related to the presence of protein show up, the amide I and amide II bands at 1646 and 1533 cm^{-1}, respectively. Observed bands at 587 and 585 cm^{-1} were attributed to Fe–O and Fe–OH stretching vibrations in the COOH MNPs and Ab MNPs, respectively. These results confirm that the MNPs were successfully conjugated with antibodies.

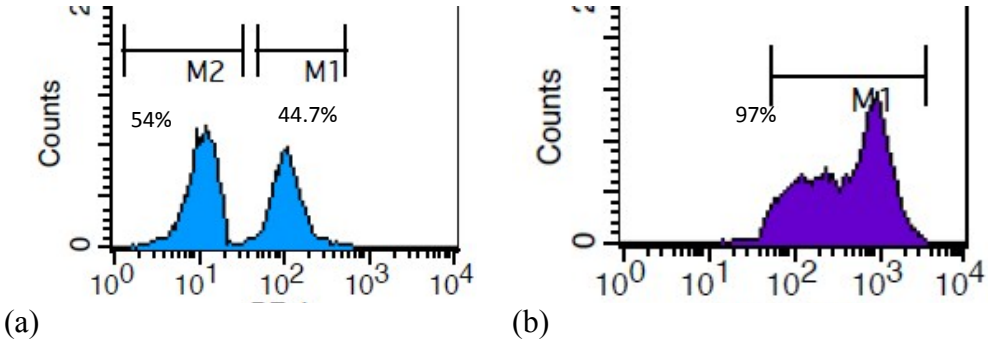
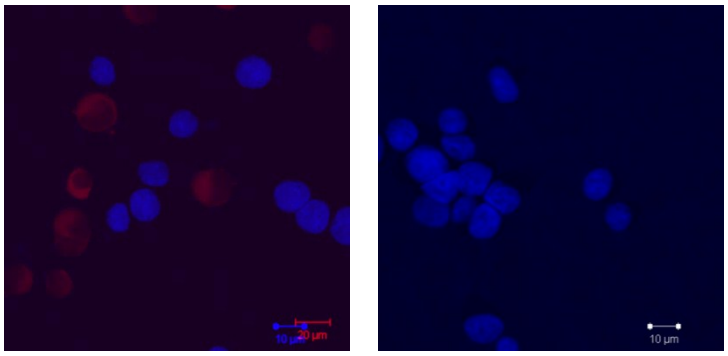


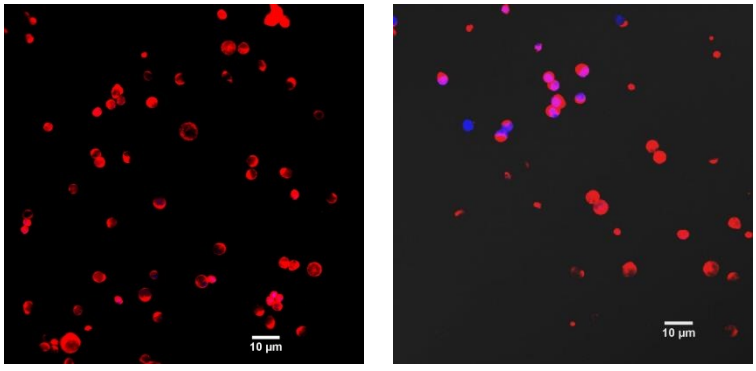
Figure S2 Specific cell isolation using Ab MNPs or COOH MNPs. (a) cell sorting by COOH MNPs (b) cell sorting by Ab MNPs. Two kinds of cells (BCRC 60427 and Ramos lymphoma)

were incubated with (a) COOH MNPs (50 μL) or (b) Ab MNPs (50 μL). The targeted cells were isolated with a magnet and washed three times. The results are expressed as the mean and standard deviation from two experiments, (a) 52 ± 2.8% lymphoma cells and 46.3 ± 2.3% hybridoma cells were isolated by COOH MNPs. (b) 97.5% ± 0.7% lymphoma cells were isolated by Ab MNPs.



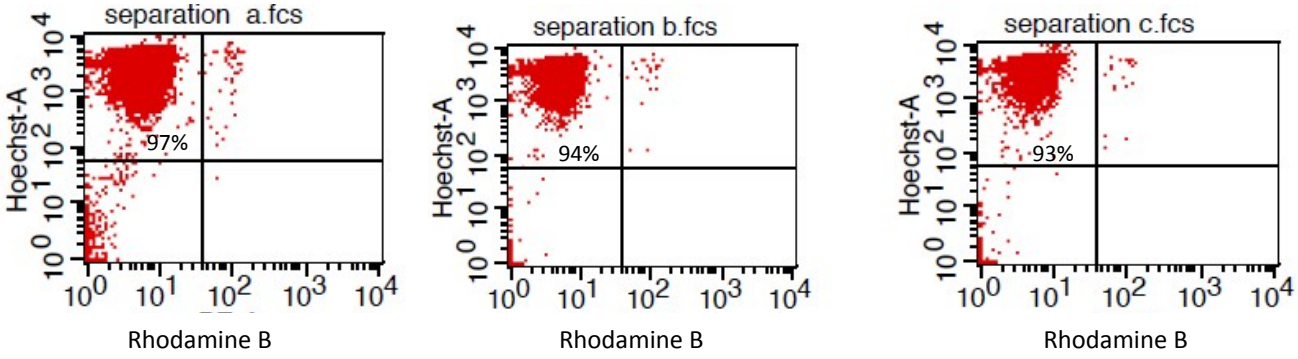
(a) (b)

Figure S3 Confocal image of specific cell isolation using Ab MNPs or COOH MNPs. (a) cell binding by COOH MNPs (b) cell binding by Ab MNPs. Two kinds of cells (BCRC 60427 and Ramos lymphoma) were incubated with (a) COOH MNPs (50 μL) or (b) Ab MNPs (50 μL). The targeted cells were isolated with a magnet and washed three times. Figure 4 a and b shows the selection results from the mixed cells by using Ab MNPs or COOH MNPs after magnetic isolation. Confocal images of CD20 positive lymphocyte cells (in blue color) or CD20 negative cells (in red color) were obtained after separated by two kinds of MNPs.



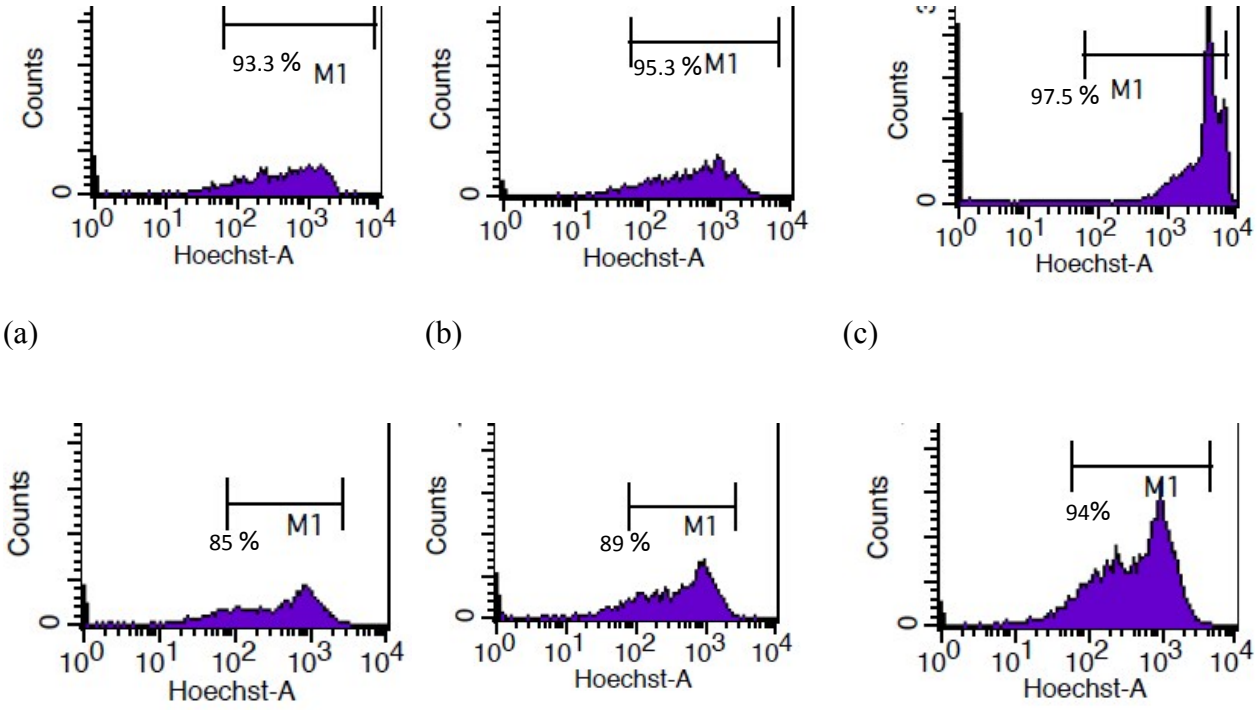
(a) (b)

Figure S4 Confocal images of cells after isolation by Ab MNPs (a) BCRC 60427, and (b) HaCaT cells. CD20 positive lymphoma cells (in blue color) or CD20 negative cells (hybridoma and HaCaT, in red color) were mixed and incubated with Ab MNPs for 20 minutes. After magnetic separation, red fluorescence of CD20 free cells was analyzed.



(a) (b) (c)

Figure S5. Flow cytometric analysis for separation efficacy using AB MNPs in different cell mixtures. (a) lymphoma and hybridoma, (b) lymphoma and CHO cells and (c) lymphoma and HaCaT. 30 µg/mL Ab MNPs were added to each cell samples and incubated for 20 minutes at 4°C. The results are obtained as the mean (n=2) and standard deviation of two determinations, 96.5 ± 0.7% (a), 94.5 ± 0.7% (b) and 94.8 ± 2.6% (c).



(a) (b) (c)

(d) (e) (f)

Figure S6 Dose-dependent capture efficacy by two kinds of Ab MNPs. CD20 positive cells (BCRC 60252) and CD20 negative cells (BCRC 60427) were isolated by using high Ab conjugated or low Ab conjugated MNPs at different concentrations. (a-c) High Ab conjugated MNPs (15.86μg Ab/mg MNPs), and (d-f) low Ab conjugated MNPs (8.07 μg Ab/mg MNPs) at 10, 30, 50 μg/mL MNPs. The mean and standard deviations (n=2) were 94.2 ± 1.3% (a), 96% ± 0.92 (b), 98 ± 0.68% (c) for high Ab conjugated MNPs and 86.5 ± 2.1% (d), 94.47 ± 5.5% (e), and 93.6 ±1.0% (f) for low Ab conjugated MNPs.

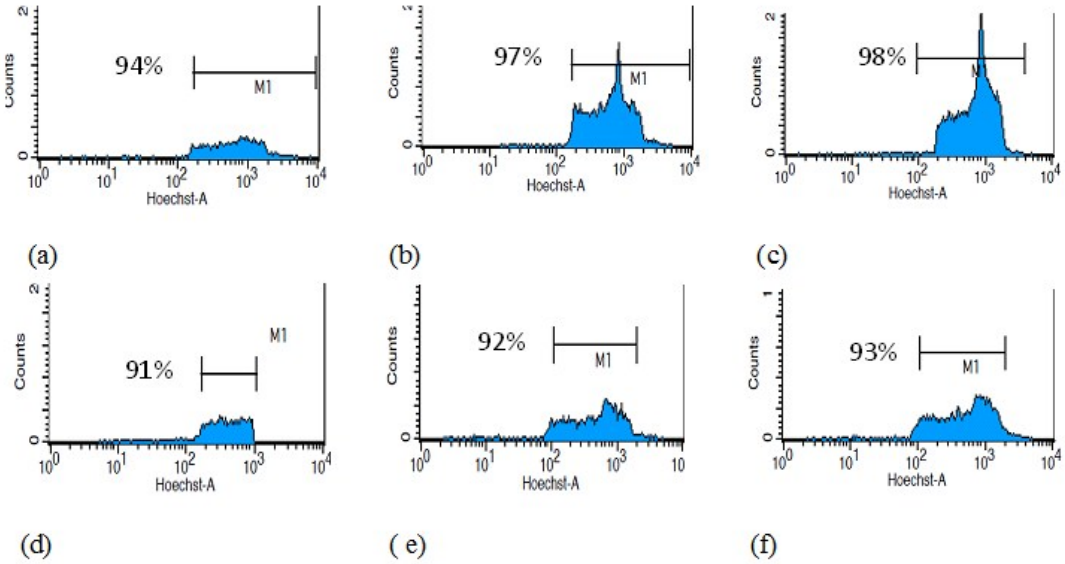


Figure S7. Dose-dependent cells isolation of the Ab MNPs. The percentage in the each figure represents the separation efficacy. CD20 positive cells (BCRC 60252) and CD20 negative cells (CHO cells) were incubated with high Ab conjugated and low Ab conjugated MNPs at different concentrations. (a-c) 10, 30, 50 μg/mL of high Ab conjugated MNPs (15.86±0.7 μg Ab per mg MNPs), and (d-f) 10, 30, 50 μg/mL of low Ab conjugated MNPs (8.07±0.02 μg Ab per mg MNPs).

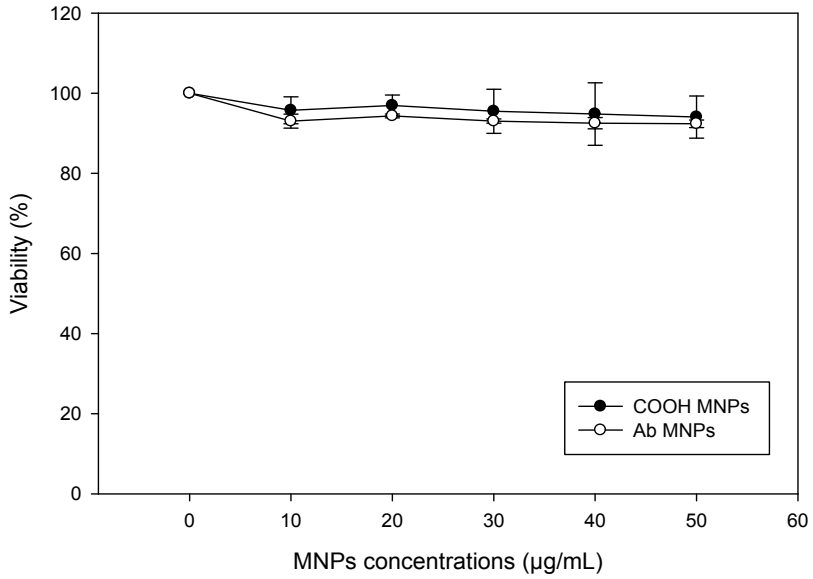
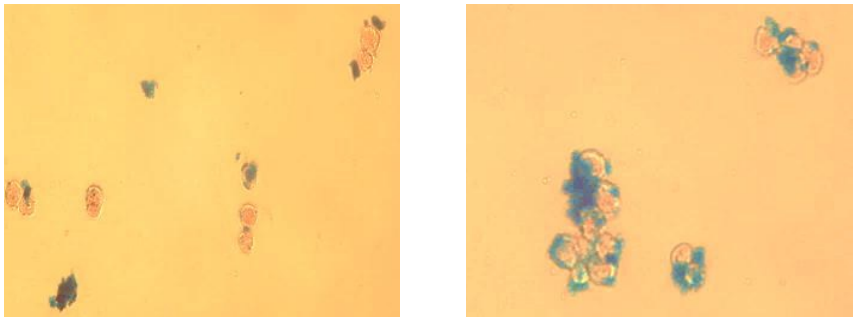


Figure S8 Biocompatibility of Ab MNPs in CHO cell line evaluated by MTT assay with different Ab MNPs concentrations ranging from 10-50 μg/mL for 24 hours. MTT assay shows that Ab MNPs do not exert toxic effect on CHO cells even at a high dosage of 50 μg/mL, suggesting that it is applicable in vivo applications. The results are the average of three replicates. The coefficient of variation (SD/mean) is around 7%.



(a) (b)

Figure S9 Prussian blue staining and quantification of intracellular iron content. Prussian blue staining (a and b) images for CD20 positive lymphoma cells (BCRC 60252) with (a) COOH MNPs, and (b) Ab MNPs for 1 hour. (Objective magnification: × 40).