Electronic Supplementary Information

Development of a phos-tag-based fluorescent biosensor for sensitive detection of protein kinase in cancer cells

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SUPPLEMENTARY RESULTS

Fig. S1 (A) Chemical structure of the phosphorylated peptide-DNA conjugates. (B) Structure of the biotinylated phos-tag.

Fig. S2 (A) Bond distances of phos-tag zinc (II) complex.① Red dotted line, coordination bond. (B) Detailed structure of phosphate skeleton of DNA in peptide-DNA.②
Fig. S3 (A) Cy5 fluorescence images prior to photobleaching step. Scale bar is 1 μm. (B) Intensity traces of single Cy5 fluorescence spots over time showing one photobleaching process.

**Optimization of experimental conditions**

To improve the detection sensitivity, we optimized the experimental conditions of RNase HII-actuated single-ribonucleotide repairing-mediated cycling signal amplification, including the concentration of signal probes and the amount of RNase HII. The value of $F/F_0$ was used to evaluate the experiments, where $F$ and $F_0$ are the fluorescence intensity in the presence and absence of peptide-DNA conjugates, respectively. As shown in Fig. S4A, the $F/F_0$ value enhances with the increasing concentration of signal probe from 1 to 10 nM, and reaches the maximum at 10 nM. Thus, 10 nM signal probe is used in subsequent experiments. We further optimized the amount of RNase HII (Fig. S4B). The $F/F_0$ value improves with the increasing amount of RNase HII from 0.05 to 0.5 U, followed by decrease beyond the amount of 0.5 U. Thus, 0.5 U of RNase HII is used in subsequent experiments.
Fig. S4 (A) Variance of the $F/F_0$ value with different concentration of signal probes. (B) Variance of the $F/F_0$ value with different amounts of RNase HII. Error bars show the standard deviation of three experiments.
Fig. S5 Fluorescence images of phos-tag-based fluorescent biosensor as a function of PKA concentration.

The red color represents the signal of Cy5. The scale bar is 5 μm.
**Fig. S6** Fluorescence images of phos-tag-based fluorescent biosensor in response to 100 nM Aurora B, 0.5 U/μL AK, 0.5 U/μL PK, 0.5 U/μL ALP, 100 nM Akt 1, 0.5 U/μL UDG, 10 μg/mL BSA, and 1 U/μL PKA, respectively. The red color represents the signal of Cy5. The scale bar is 5 μm.
Reference
