Supporting Information for

# Simple and label-free strategy for terminal transferase assay using a personal glucose meter

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## Experimental section

### *Materials*

The personal glucose meter (PGM) was purchased from Accu-Chek (Roche, Basel, Switzerland). Cerium (IV) oxide nanoparticle ($CeO_2$ NP), sodium acetate, glucose, and SYBR Gold were purchased from Sigma-Aldrich (St. Louis, MO, USA). The deoxynucleoside triphosphate (dNTP) and terminal transferase (terminal deoxynucleotidyl transferase, TdT) were purchased from New England Biolabs Inc. (Beverly, MA, USA). TdT primer (TP, 5'-GGA CCA TTA CCA GAC AGT GTT A-3') was synthesized and purified with high performance liquid chromatography by Bioneer® (Daejeon, Korea). Human blood was purchased from ZenBio Inc. (Research Triangle Park, NC, USA). Ultrapure DNase/RNase-free distilled water (DW) purchased from Bioneer® was used in all experiments. All chemicals used in this study were of analytical grade.

### *TdT activity assay*

First, the 20 μL TdT reaction solution composed of 13 μL DW, 1 μL dNTP (10 mM each), 1 μL TP (20 μM), 1 μL TdT at varying concentrations, and 4 μL TdT reaction buffer (100 mM Tris-acetate (pH 7.9), 50 mM magnesium acetate, 250 mM potassium acetate, and 1.25 mM cobalt (II) chloride) was incubated at 37 ˚C for 60 min using S1000™ thermal cycler (Bio-Rad, CA, USA). Next, the $CeO_2$ NP reaction solution composed of 4 μL DW, 4 μL sodium acetate

buffer (1 M, pH 4.2), and 4 μL $CeO_2$ NP (0.5 wt%) was added to 20 μL TdT reaction solution, which was incubated at room temperature for 2 min. Finally, 8 μL glucose (300 mM) was added into the afore-mentioned reaction solution, which was further incubated at room temperature for 1.5 min. The resulting glucose level was measured by PGM.

***Spike-and-recovery test***

TdT at varying concentrations was spiked into the 5% human blood, which was analyzed by the same procedure described in 'TdT activity assay'. To determine the TdT concentration spiked into the 5% human blood, the calibration curve was first drawn with a set of standards containing known concentrations of TdT and the spiked TdT concentration was then determined based on the calibration curve.

***Investigation of DNA binding-induced aggregation of $CeO_2$ NP***

The crystalline nature of the employed $CeO_2$ NP was examined by using SmartLab X-ray diffractometer (Rigaku, Tokyo, Japan) with monochromatic Cu-Kα radiation. The transmission electron microscopy (TEM) and energy-dispersive X-ray spectroscopy (EDS) images were obtained by using Cs-corrected STEM (JEM-ARM200F) in National NanoFab Center (NNFC, Daejeon, Korea). Samples for TEM and EDS analysis were prepared as follows: 15 μL sample was casted onto the copper grid (300 mesh) with a lacey carbon film (LC300-CU) purchased

from Electron Microscopy Sciences (Hatfield, PA, USA), which was then dried at room temperature overnight. The zeta potential analysis was performed by using Zetasizer (Malvern, PA, USA). For the measurement of zeta potential of $CeO_2$ NP, 1 mL sample composed of 0.5 mL $CeO_2$ NP (1 mg/mL) and 0.5 mL TdT reaction solution was added in Zetasizer capillary cell and then scanned three times to obtain average zeta potential values.

***Gel electrophoresis***

10 μL TdT reaction solution was first mixed with 2 μL loading buffer (6X) purchased from Bioneer®, which was then loaded on 15% polyacrylamide gel. Next, the gel electrophoresis was conducted at 120 V for 100 min by using 1X TBE as the running buffer. After staining with SYBR Gold, a gel image was taken utilizing Gel Doc™ EZ Imager (Bio-Rad, CA, USA).

**Table S1.** Comparison of this strategy with the existing TdT assays.

| Material/Method | Detection limit (U/mL) | Limitations | Reference |
|---|---|---|---|
| DNA-templated silver nanocluster | 0.318 | Synthesis of metal nanoparticle/nanocluster | 1 |
| DNA-templated copper nanoparticle | 3.75 | | 2 |
| Graphene oxide-supported DNA-templated silver nanocluster | 0.08 | | 3 |
| Randomly arrayed G-quadruplexes | 0.394 | Bulky and specialized instrument | 4 |
| Iridium (III)-based i-motif probe | 0.25 | | 5 |
| Iron (III)-quenched boron-dipyrromethene-adenosine triphosphate | 0.64 | | 6 |
| GOx-mimicking activity of $CeO_2$ NP | 0.7 | - | This work |

**Table S2.** CV values of the samples containing TdT at varying concentrations in the range from 0 to 500 U/mL, which are defined as S/M × 100 where M and S are mean and standard deviation of the resulting PGM signals, respectively. The concentrations of dNTP, TP, $CeO_2$ NP, and glucose were 0.25 mM each, 0.5 μM, 0.05 wt%, and 60 mM, respectively.

| TdT concentration (U/mL) | M | S | CV (%) |
|---|---|---|---|
| 0 | 59.00 | 1.73 | 2.94 |
| 10 | 63.00 | 1.73 | 2.75 |
| 20 | 71.33 | 3.21 | 4.51 |
| 40 | 89.00 | 1.00 | 1.12 |
| 60 | 101.67 | 4.73 | 4.65 |
| 80 | 119.33 | 3.21 | 2.69 |
| 100 | 135.67 | 3.21 | 2.37 |
| 250 | 148.67 | 9.07 | 6.10 |
| 500 | 155.67 | 4.04 | 2.60 |

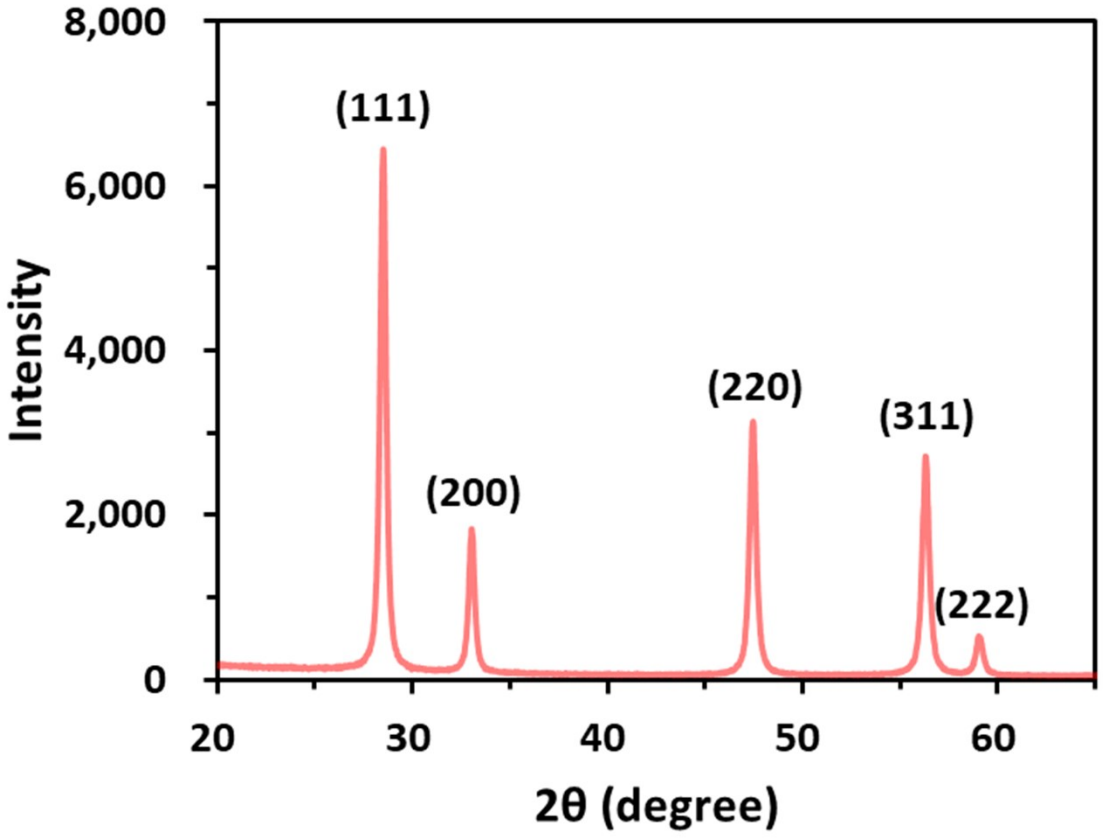


**Fig. S1.** XRD spectrum of the employed $CeO_2$ NP.

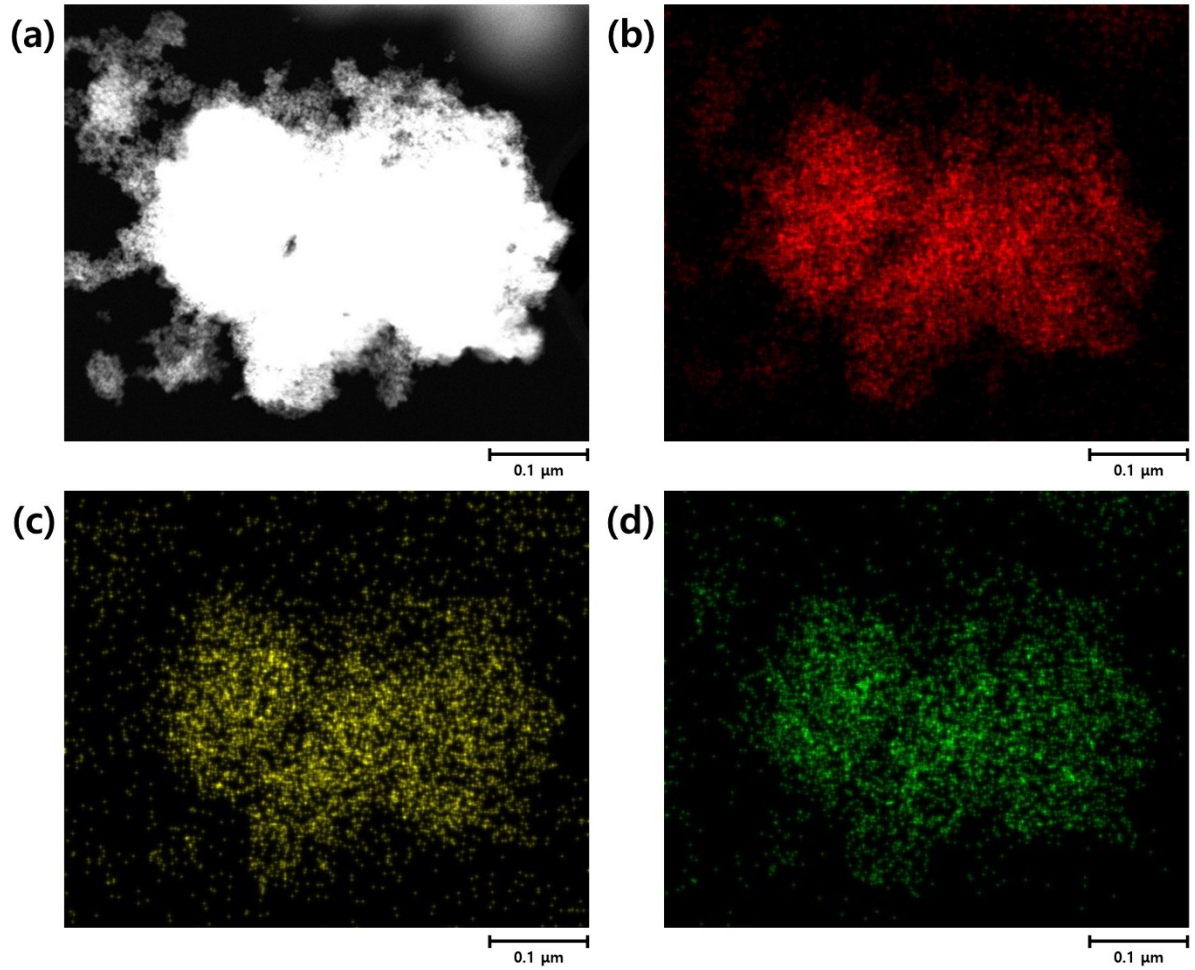


**Fig. S2.** The confirmation of EP binding onto $CeO_2$ NPs. (a) TEM image of $CeO_2$ NPs in the presence of TdT that produces EP and corresponding EDS images of (b) Ce, (c) N, and (d) P. The concentrations of dNTP, TP, $CeO_2$ NP, glucose, and TdT were 0.25 mM each, 0.5 μM, 0.05 wt%, 60 mM, and 100 U/mL, respectively.

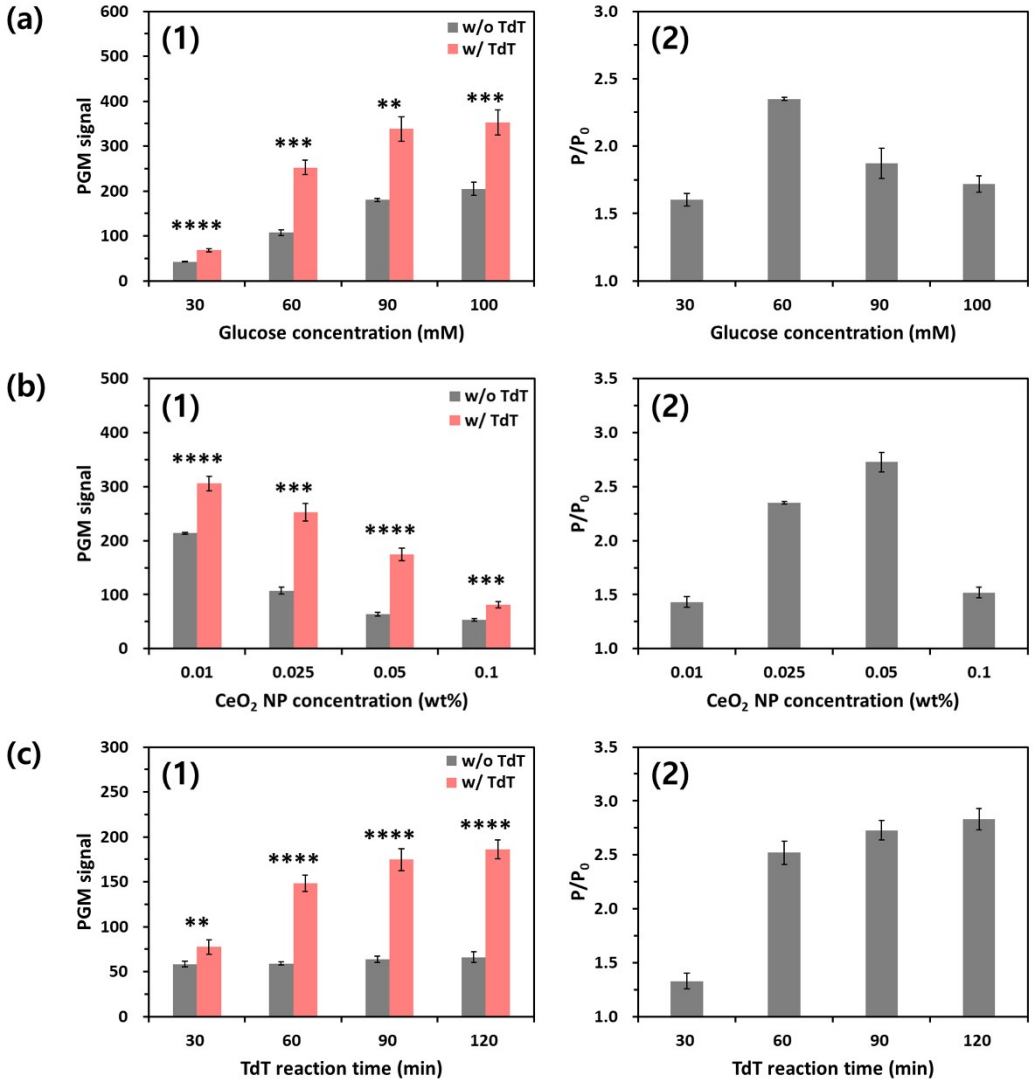


**Fig. S3.** Optimization of reaction conditions. (a) glucose concentration, (b) $CeO_2$ NP concentration, and (c) TdT reaction time were optimized by (1) measuring PGM signals and (2) calculating PGM signal change defined as $P/P_0$ where $P_0$ and P are PGM signals in the absence and presence of TdT, respectively, from the samples at varying reaction conditions. The concentrations of dNTP, TP, and TdT were 0.25 mM each, 0.5 μM, and 250 U/mL, respectively. Asterisks indicate the different p-value of the sample containing TdT compared to the negative control without TdT (* > 0.05, ** < 0.05, *** < 0.01, and **** < 0.001).

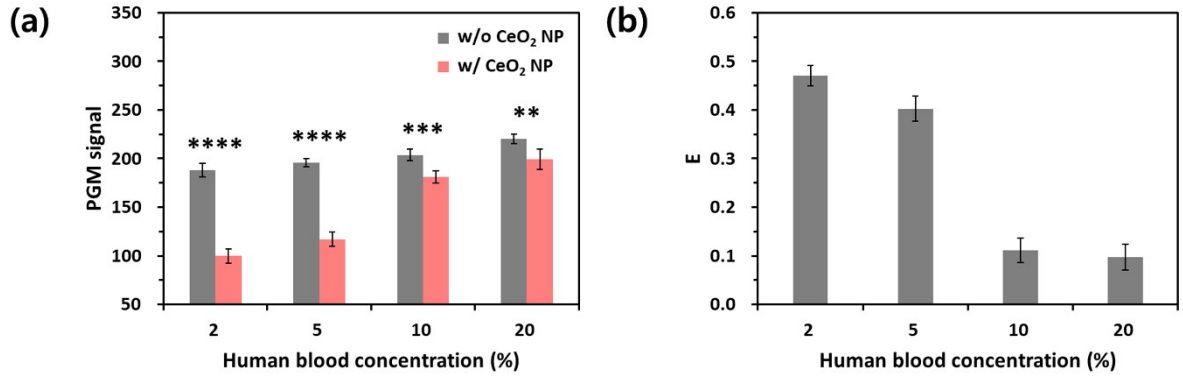


**Fig. S4.** The effect of the human blood on $CeO_2$ NP-catalyzed glucose oxidation. (a) PGM signals and (b) glucose oxidation efficiency (E) of the samples containing the human blood at varying concentrations, which is defined as $(P_0 - P)/P_0$ where $P_0$ and P are PGM signals in the absence and presence of $CeO_2$ NP, respectively. The concentrations of $CeO_2$ NP and glucose were 0.05 wt% and 60 mM, respectively. Asterisks indicate the different p-value of the sample containing TdT compared to the negative control without TdT (* > 0.05, ** < 0.05, *** < 0.01, and **** < 0.001).

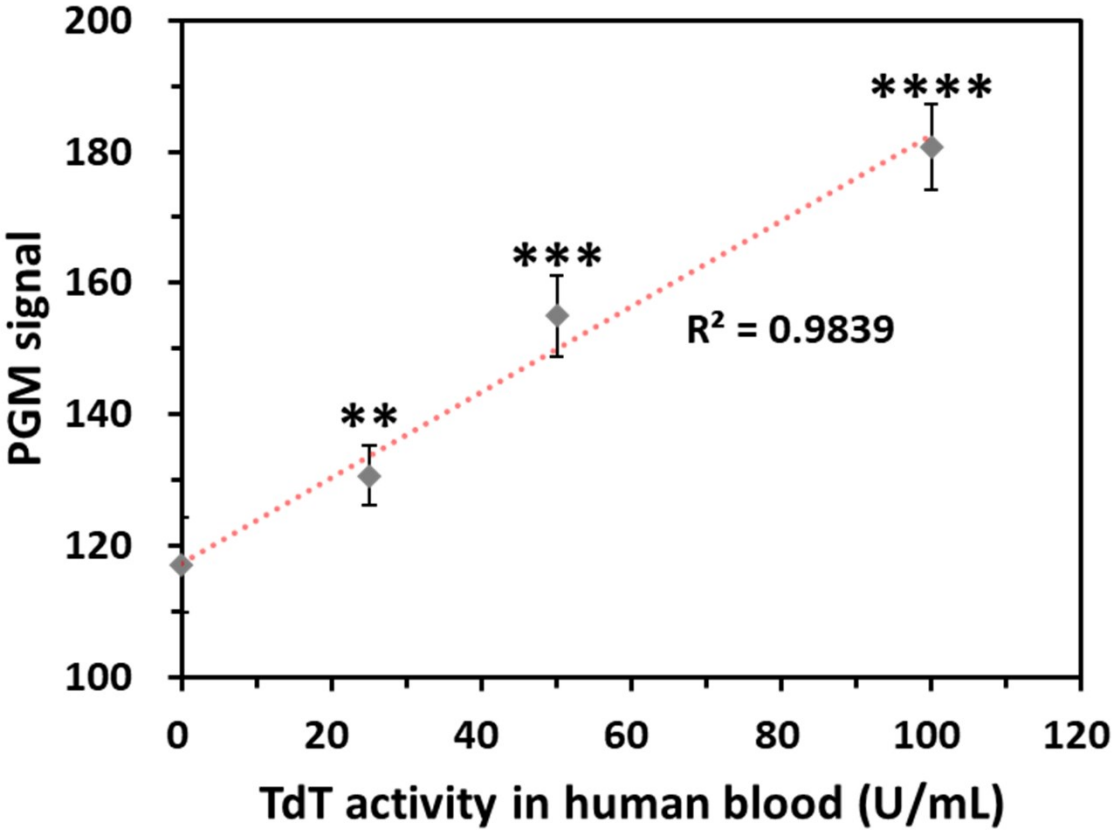


**Fig. S5.** PGM signals from the samples containing varying TdT activities in human blood. The concentrations of dNTP, TP, $CeO_2$ NP, glucose, and human blood were 0.25 mM each, 0.5 μM, 0.05 wt%, 60 mM, and 5%, respectively. Asterisks indicate the different p-value of the sample containing TdT compared to the negative control without TdT (* > 0.05, ** < 0.05, *** < 0.01, and **** < 0.001).

## References


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