Validation data for HEK-RepTor™ cells

https://www.invivogen.com/tet-on-hek293-reptor-cells

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Version 24A25-NJ

HEK-RepTor™ cells are designed for the Tet-on inducible expression of a protein. The absence of expression leakage has been verified in HEK-RepTor™ cells transfected with pTiGer plasmids coding for the SEAP (secreted embryonic alkaline phosphatase) or Lucia luciferase reporter proteins **(Figure 1).** SEAP and Lucia are conditionally expressed by HEK-RepTor™ SEAP and HEK-RepTor™ Lucia cells upon incubation with Doxycycline (Dox), in a dose-dependent manner **(Figure 2).** Additionally, the conditional expression of a cell-damaging protein has been assessed in a functional assay, using HEK-RepTor™ GSDMD-Nter cells. These cells were transfected with a pTiGer plasmid coding the N-terminal domain of gasdermin D (GSDMD-Nter). GSDMD-Nter expression starts 2 hours post-treatment with Dox as assessed by Western blot **(Figure 3)**. The subsequent GSDMD-Nter-mediated cell death has been monitored 24 hours post-treatment using the lactate dehydrogenase (LDH) assay **(Figure 4)**.

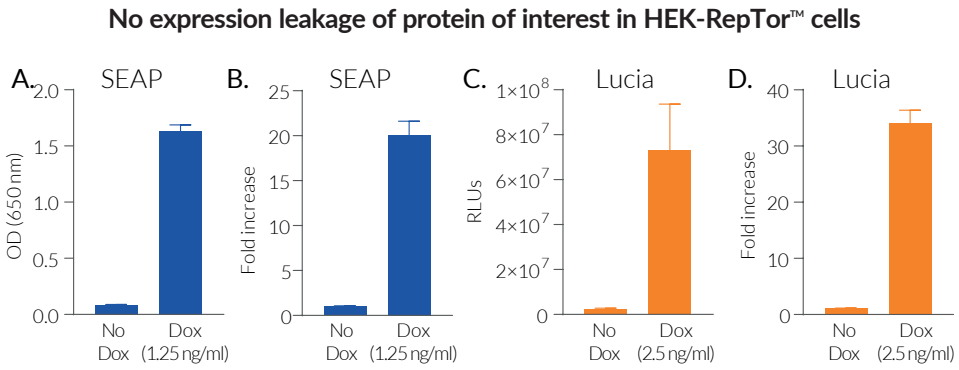

Figure 1: Expression of SEAP or Lucia reporter proteins in transfected HEK-RepTor™ cells. HEK-RepTor™ cells were transfected with the gene coding for SEAP (secreted embryonic alkaline phosphatase) or Lucia luciferase cloned into a pTiGer plasmid. The cells were then treated, or not, with Doxycycline (Dox) at 1.25 or 2.5 ng/ml for 24 hours. **(A, B)** The SEAP activity in the supernatant of HEK-RepTor™ SEAP cells was assessed using QUANTI-Blue™ detection reagent. The data is shown as **(A)** OD at 650 nm and **(B)** fold increase (mean +SEM). **(C, D)** The Lucia activity in the supernatant of HEK-RepTor™ Lucia cells was assessed using QUANTI-Luc™ 4 Lucia/Gaussia detection reagent. The data is shown as **(C)** relative light units (RLUs) and **(D)** fold increase (mean +SEM).

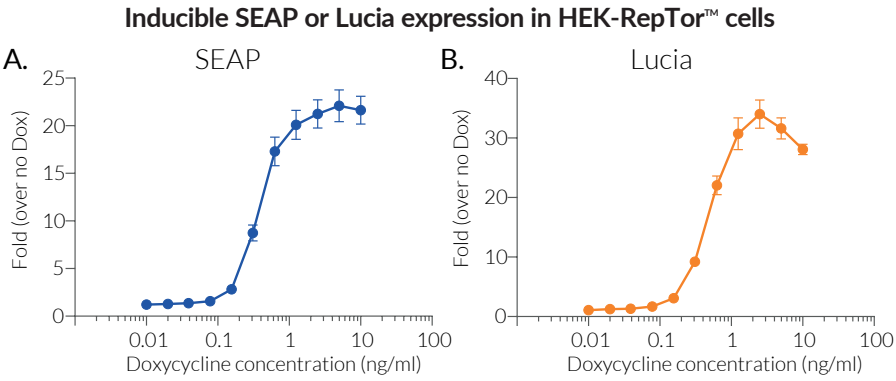

Figure 2: Dose-dependent Doxycycline-mediated expression of SEAP or Lucia in HEK-RepTor™ cells. HEK-RepTor™ cells were transfected or not with the gene coding for SEAP (secreted embryonic alkaline phosphatase) or Lucia luciferase cloned into a pTiGer plasmid. HEK-RepTor™ SEAP cells and HEK-RepTor™ Lucia cells were incubated with increasing concentrations of Doxycycline. After 24 hours, the SEAP or Lucia activity in the supernatant was assessed using **(A)** QUANTI-Blue™ or **(B)** QUANTI-Luc™ 4 Lucia/Gaussia, respectively. The data is shown as fold induction over no Dox treatment (mean + SEM).

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Inducible GSDMD-Nter expression in HEK-RepTor™ cells

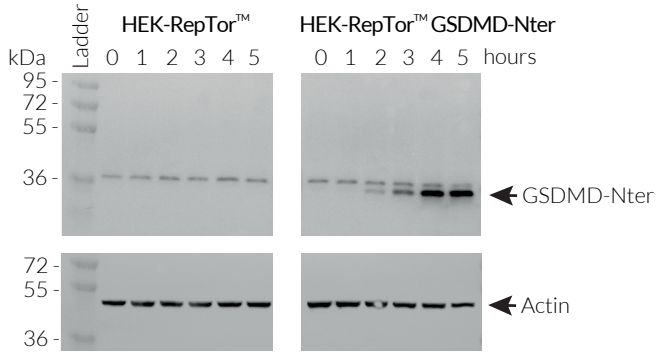

Figure 3: Doxycycline-mediated expression of GSDMD-Nter in HEK-RepTor™ cells. HEK-RepTor™ cells were transfected or not with the gene coding for human GSDMD-Nter cloned into a pTiGer plasmid. HEK-RepTor™ and HEK-RepTor™ GSDMD-Nter cells were then treated with Doxycycline (Dox) at 1 ng/ml. Cell lysates were collected at 1 to 5 hours after Dox addition. They were analyzed by Western blot using an anti-cleaved N-terminal GSDMD antibody (Abcam #215203) followed by an HRP-conjugated anti-rabbit secondary antibody (Southern Biotech #4050-05), and anti-Actin (Invitrogen #AM4302) antibodies, followed by an HRP-conjugated anti-mouse secondary antibody (Southern Biotech #1034-05).

Conditional GSDMD-mediated cell death in HEK-RepTor™ GSDMD-Nter cells

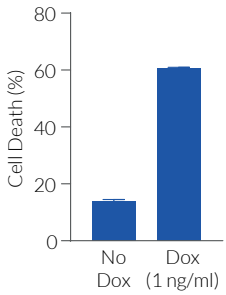

Figure 4: Doxycycline-mediated cell death in HEK-RepTor™ GSDMD-Nter cells. HEK-RepTor™ cells were transfected with the gene coding for human GSDMD-Nter cloned into a pTiGer plasmid. HEK-RepTor™ GSDMD-Nter cells were then treated with Doxycycline (Dox) at 1 ng/ml for 24 hours. The cell death mediated by the induced expression of GSDMD-Nter was assessed using the lactate dehydrogenase (LDH) assay. Data is shown as percentage of cell death (mean + SEM).