



### Knock-down rate: 67%

**Lanes:** 1. HeLa cells transfected with Renilla Luciferase esiRNA (RLUC, Cat.No. EHURLUC) as negative control. 2. HeLa cells transfected with UBL5 esiRNA (Cat.No. EHU122201).

**Quantitative-Immunoblotting:** Lysates were separated on SDS-Page and probed with mouse anti-GFP (1:3000) (Roche) and goat anti-GAPDH (1:7000) (Acris) antibodies. The signal intensities of indicated bands were quantified using an Odyssey infrared imaging system (Li-COR) and normalized to the GAPDH signal. At 72h post transfection, a 67% UBL5-GFP protein reduction was measured between the negative control RLUC and the sample treated with UBL5 esiRNA.

**HeLa cells:** UBL5 was LAP tagged (including GFP) on a bacterial artificial chromosome (BAC) and stably integrated into the genome of HeLa cells (MCB: ky\_6120). The BAC preserves the genomic context of the gene, thereby ensuring near physiological expression (Poser I. et al Nat Methods. 2008 May;5(5):409-15).