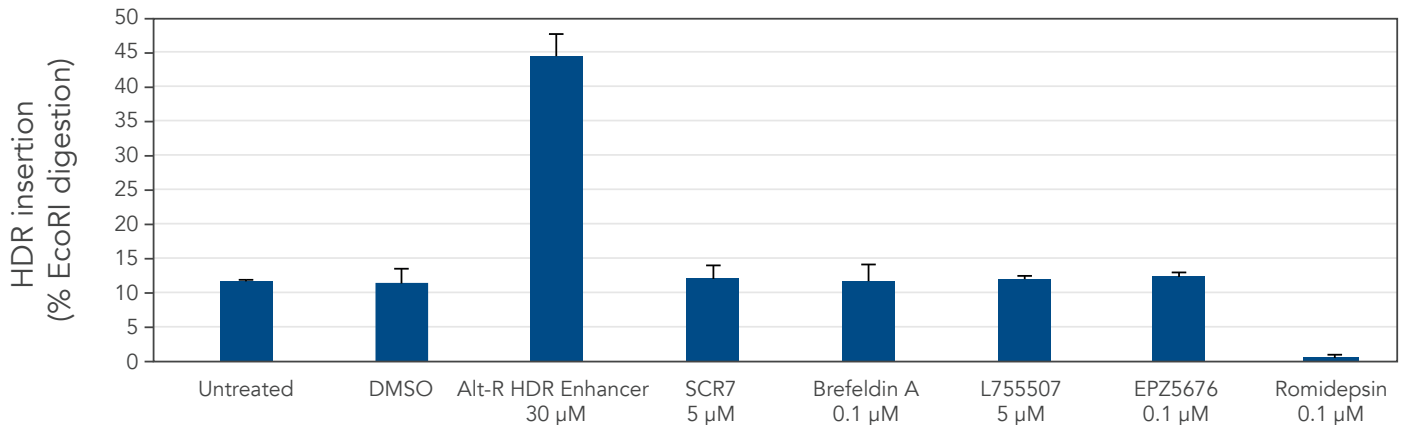




## Alt-R HDR ENHANCER IMPROVES HDR EFFICIENCY SEVERAL FOLD

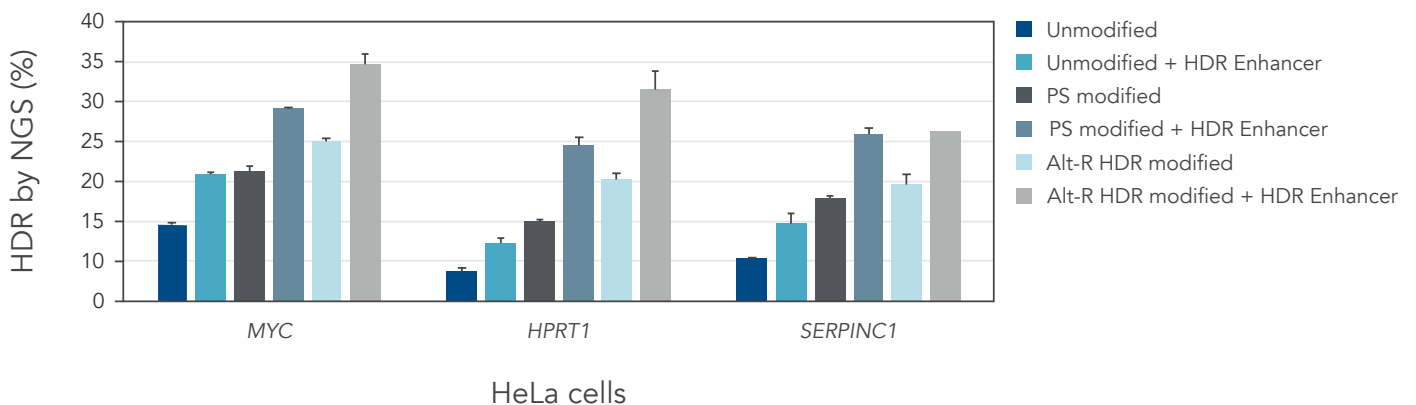
We tested several possible chemical compounds for their ability to improve HDR efficiency. Our Alt-R HDR Enhancer more than tripled the rate of HDR, greatly outperforming all other compounds (Figure 2).



**Figure 2. Alt-R HDR Enhancer improves HDR efficiency by more than 3-fold compared to other tested compounds.** RNP complex (4 µM) targeting human *HPRT1* and 3 µM modified HDR donor oligos designed to insert an EcoRI restriction site were delivered together into Jurkat cells by electroporation using the 4D-Nucleofector™ System (Lonza). The RNP complex comprised Alt-R S.p. Cas9 Nuclease V3 complexed with Alt-R CRISPR-Cas9 crRNA and tracrRNA. Immediately after electroporation, cells were cultured in media containing either 30 µM Alt-R HDR Enhancer or a recommended dose of small molecules (SCR7, Brefeldin A, L755507, EPZ5676, and romidepsin) proposed to improve HDR rates. Similar volumes of 1X PBS or DMSO were used as the untreated and negative controls, respectively. Genomic DNA was isolated 72 hours after electroporation, and HDR efficiency was measured by EcoRI cleavage of PCR amplicons from the target region in the *HPRT1* gene.

## Alt-R HDR MODIFIED DONORS AND Alt-R HDR ENHANCER LEAD TO THE HIGHEST HDR RATES

We investigated whether combining Alt-R HDR Enhancer with Alt-R modifications of HDR donor oligos would improve HDR rates further. Our data confirmed that this works well, leading to the highest HDR rates (Figure 3).



**Figure 3. Alt-R HDR modified donors and Alt-R HDR Enhancer have an additive effect on HDR improvement.** RNP complexes (2 µM) targeting 3 genomic loci along with 0.5 µM single-stranded HDR donor oligo were delivered to HeLa cells by electroporation using the 4D-Nucleofector™ System (Lonza). The RNP complex comprised Alt-R S.p. HiFi Cas9 Nuclease V3 complexed with Alt-R CRISPR-Cas9 crRNA and tracrRNA. Unmodified, PS modified, or Alt-R HDR modified donor templates were used. Immediately after electroporation, cells were plated in media with or without 30 µM Alt-R HDR Enhancer. Genomic DNA was isolated 48 hours after electroporation, and HDR efficiency was measured by amplicon sequencing on an Illumina™ MiSeq™ system (v2 chemistry, 150 bp paired-end reads).

Design and order your HDR donor templates and associated Cas9 guide RNAs for genome editing of human, mouse, rat, zebrafish, or *C. elegans* targets with the Alt-R HDR Design Tool at [www.idtdna.com/HDRdesigntool](http://www.idtdna.com/HDRdesigntool).

If you already have your HDR donor design(s), order them at [www.idtdna.com/HDRdonoroligos](http://www.idtdna.com/HDRdonoroligos) and take advantage of our HDR donor oligos specifically built for successful homology-directed repair.