

# Alt-R™ HDR Enhancer Protein

A protein-based, proprietary HDR Enhancer engineered for efficiency without compromising cell viability or genomic integrity



## Excellent performance

Up to 2X increase in HDR rates in established and primary cell lines (i.e. HEK293, iPSCs, HSPCs)



## Precision editing

Without increasing off-target effects, translocations, or compromising cell viability



## Seamless integration

Into existing workflows available as RUO and CGMP (coming soon) grades

## Boost your edits, fast-track your breakthrough

The Alt-R HDR Enhancer Protein is a proprietary, mechanism-specific reagent that increases HDR efficiency by inhibiting 53BP1, a key regulator of DNA repair pathway choice. Unlike small-molecule enhancers that block the NHEJ (non-homologous end joining) pathway this protein has minimal effects on NHEJ pathway while promoting HDR. Validated across challenging primary cells, including iPSCs and HSPCs, the enhancer provides consistent, reproducible performance. Alt-R HDR Enhancer Protein offers increased HDR with minimal impact on unintended editing and minimal cytotoxicity, making it ideal for both research and therapeutic workflows (CGMP grade coming soon).

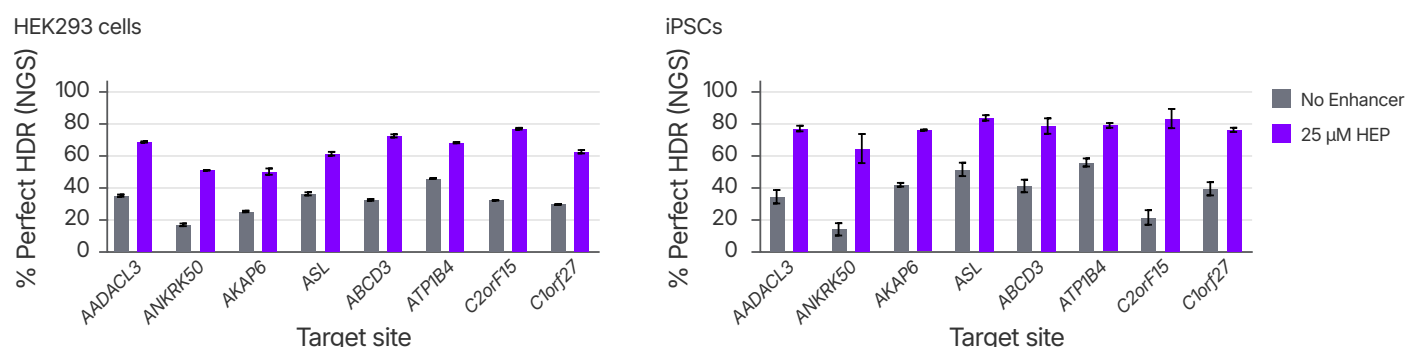
## Benefits

1. Significant HDR boost—up to 2X higher HDR efficiency in multiple cell types (i.e., HEK293, iPSCs, HSPCs)
2. Broad compatibility—works across diverse CRISPR-Cas systems and donor templates
3. No added off-targets or translocations—editing analysis confirms no additional Cas9 off-target sites or translocations
4. High viability—maintains cell viability across a wide concentration range
5. Workflow integration—simple, plug-and-play addition to existing CRISPR workflows
6. Translational ready—RUO format available now; CGMP grade coming soon!

## Performance

### Alt-R HDR Enhancer Protein improves HDR efficiency in hard-to-edit cells

Alt-R HDR Enhancer Protein shows a consistent increase in perfect HDR of a 6 bp insertion in two different cell lines (HEK293 and iPSCs) at eight genomic loci.



**Figure 1. Alt-R HDR Enhancer Protein (HEP) increases rates of HDR in multiple cell types.** RNP complexes comprised of Cas9 and sgRNA targeting various loci were delivered to HEK293 cells at 2 µM or iPSCs (Coriell Institute) at 4 µM, with 2 µM ssDNA donor (Alt-R HDR Donor Oligo), with or without Alt-R HDR Enhancer Protein using the 4D-Nucleofector System (Lonza). HDR was assessed using NGS. Error bars indicate SD,  $n = 3$ .

**RUO**

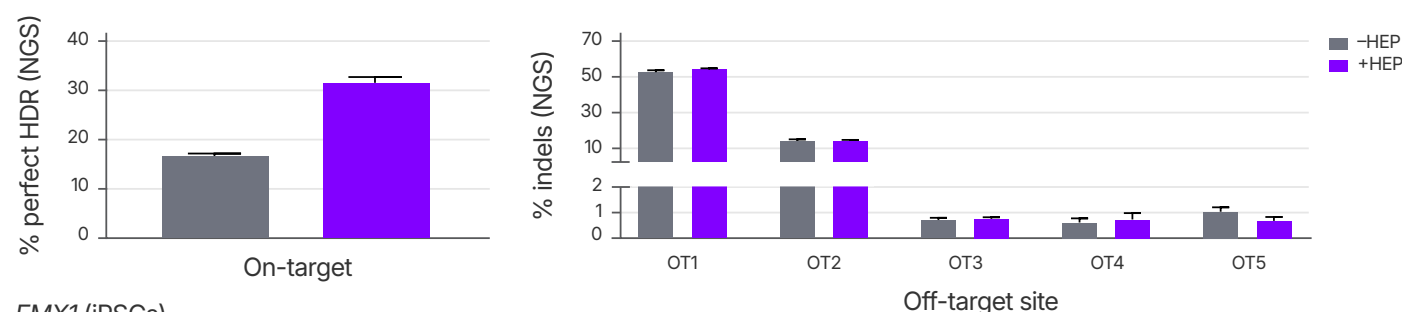
For Research Use Only. Not for use in diagnostic procedures.

[idtdna.com](http://idtdna.com)

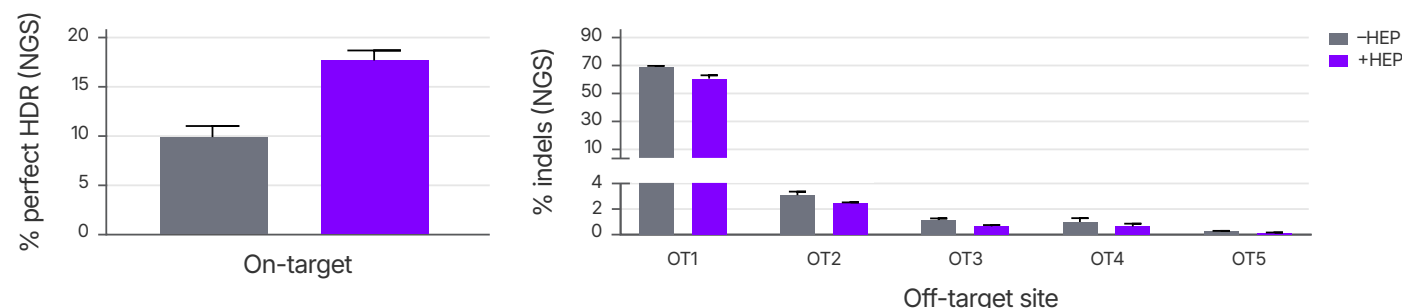
## Alt-R HDR Enhancer Protein improves on-target editing without increasing off-target indels

Alt-R HDR Enhancer Protein improves perfect HDR of a 6 bp insertion in established (HEK293) and primary (iPSCs) cell types while maintaining low off-target indel frequencies.

### EMX1 (HEK293 cells)



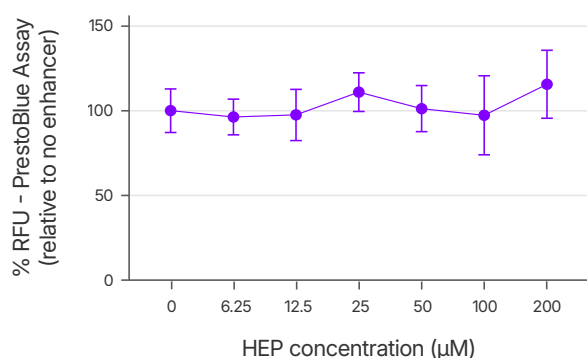
### EMX1 (iPSCs)



**Figure 2. Use of Alt-R HDR Enhancer Protein does not increase indels at off-target sites.** 2  $\mu$ M RNP comprising Cas9 and sgRNA targeting EMX1, 2  $\mu$ M ssDNA Alt-R HDR Donor Oligo, and 0 or 25 HEP (HEK293 cells) or 12.5  $\mu$ M uM HEP (iPSCs) were delivered using the 4D-Nucleofector System (Lonza). Editing was measured after 48 hours using rhAmpSeq™ NGS targeting the EMX1 on-target site and a set of known off-target sites. Error bars indicate SD,  $n = 3$ .

## Alt-R HDR Enhancer Protein does not induce cellular toxicity across a wide concentration range

Alt-R HDR Enhancer Protein does not reduce cell viability, confirming its suitability for use in sensitive cell types and high-efficiency editing protocols.



**Figure 3. No cytotoxicity observed with increasing doses of Alt-R HDR Enhancer Protein.** 2  $\mu$ M RNP complex targeting EMX1, 2  $\mu$ M ssDNA Alt-R HDR Donor Oligo, and varying concentrations of Alt-R HDR Enhancer Protein were delivered to HEK293 cells using the 4D-Nucleofector System (Lonza). Cell viability was measured 48 hours post-treatment using the PrestoBlue assay, with results reported as percent relative fluorescence units (RFU) compared to untreated controls. Error bars represent standard deviation (SD);  $n = 3$  biological replicates.

Product	Grade	Size	Catalog #
Alt-R™ HDR Enhancer Protein	RUO	500 $\mu$ g	10029790
	RUO	5 mg	10029801
Alt-C™ HDR Enhancer Protein	CGMP		<a href="http://www.idtdna.com/CGMP-HDR-Enhancer-Protein">www.idtdna.com/CGMP-HDR-Enhancer-Protein</a>

For more information, visit [idtdna.com/HDREnhancerProtein](http://idtdna.com/HDREnhancerProtein)



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