HuSH-29 shRNA Kinase Collection

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HuSH-29 shRNA Overview

HuSH-29 are pre-designed shRNA with genome wide coverage (human, mouse and rat) that delivers guaranteed knockdown. The same superior design is also offered for other species through Exact-shRNA.

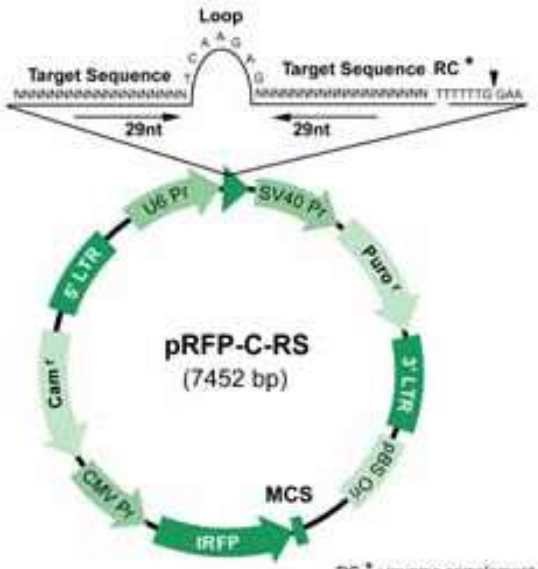
- Three retroviral vector options:
  - pRS: basic retroviral vector without fluorescence
  - pRS with GFP to monitor transfection
  - pRS with RFP, ideal for double knockdown
- Guaranteed successful gene knockdown (≥70%)
- Versatile applications: transient or stable transfection or retroviral infection
- Transfection-ready DNA using PowerPrep® HP kits

HuSH-29 shRNA cassette

The length and design of HuSH-29 hairpin is a critical improvement over the use of 21mer designs. Longer shRNA constructs appear to enter the RNAi pathway more efficiently and result in much higher potency and specificity than shorter expressed RNAi forms. In most mammalian cells, long double-stranded RNA provokes an interferon response as part of an antiviral defense. This obstacle can be overcome by using shRNA less than 30 base pairs in length, which evades the radar of the mammalian interferon response and initiates strong and specific gene silencing. By its optimal length, HuSH-29 has the advantages of improved efficacy and minimal interferon response.

pRFP-C-RS vector

pRFP-C-RS plasmid vector contains both 5’ and 3’ LTRs of Moloney murine leukemia virus (MMLV). Upon transient transfection of the plasmids into a packaging cell line, replication deficient viruses can be obtained and used to infect target cells. It also incorporates both a chloramphenicol and puromycin resistance elements for greater selection capabilities. There is an integrated turboRFP element driven by a cMV promoter to readily verify transfection efficiency.The pRFP-C-RS plasmid is also ideal for dual-gene knockdown when used alongside pGFP-V-RS vector.



Specifications of Kinase Collection

ORIGENE Technologies

- HuSH-29 shRNA kinase collection is available against human genes
- shRNA constructs targeting 512 genes plated either individually or as a pooled set
- 4 shRNA constructs provided per gene targeting different regions of the ORF
- Individually plated collection: 22 plates
  2ug/well in 96-well microtiter plates
- Constructed in pRFP-C-RS vector

Western validation with Kinase set

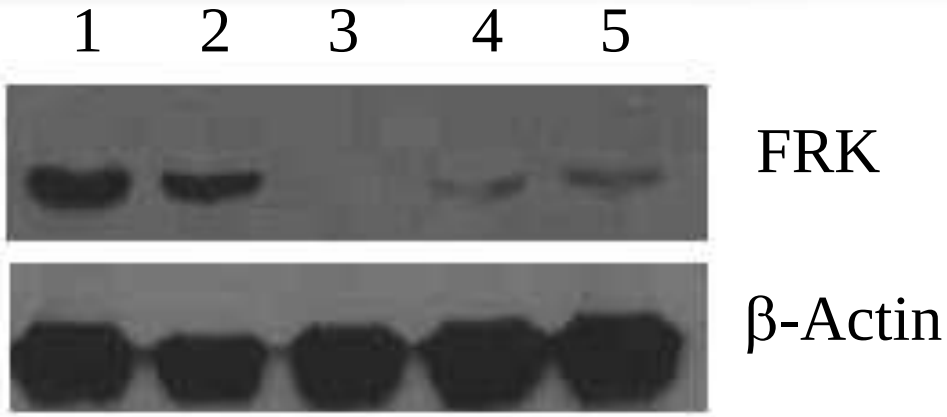
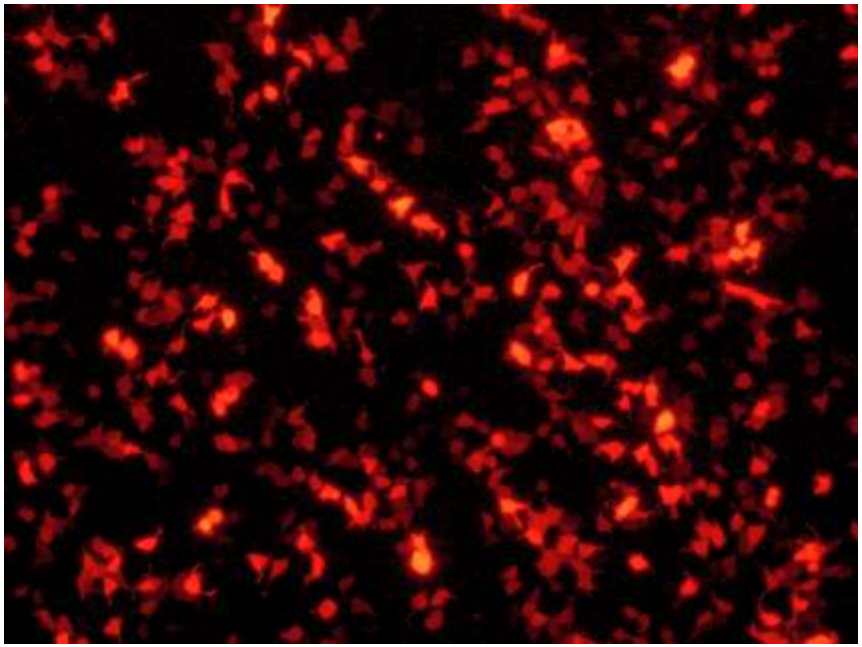


Fig. Hush shRNA constructs and corresponding TrueORF cDNA clone were co-transfected in HEK293 cells. Cell were harvested 48 hr post-transfection and western blot was performed using anti-DDK (Flag) antibody.

Lan1	Symbol	Accession	Clone SKU	RFP	shRNA Sku
1	FRK	NM_002031	RC204460		Scrambled
2	FRK	NM_002031	RC204460	TF320363	FI378669
3	FRK	NM_002031	RC204460	TF320363	FI378670
4	FRK	NM_002031	RC204460	TF320363	FI378671
5	FRK	NM_002031	RC204460	TF320363	FI378672

Transient transfection using pRFP-C-RS



pRFP-C-RS vector transiently transfected in HEK293T cells for easy monitoring of transfection efficiency.

Guarantee

OriGene guarantees that the sequences in the shRNA expression cassettes are verified to correspond to the target gene with 100% identity. For the individually plated collection, one of the four constructs at minimum are guaranteed to produce 70% or more gene expression knock-down provided a minimum transfection efficiency of 80% is achieved. To properly assess knockdown, the gene expression level from the included scramble control vector must be used in comparison with the target-specific shRNA transfected samples.

For non-conforming shRNA, requests for replacement product must be made within ninety (90) days from the date of delivery of the shRNA kit. To arrange for a free replacement of four newly designed constructs, please contact Technical Services at techsupport@origene.com. Please provide your data indicating the transfection efficiency and measurement of gene expression knockdown compared to the scrambled shRNA control.