Self-deliverable siRNA(sdRNA)

DESCRIPTION

Self-deliverable siRNA compounds are chemically modified nucleic acid duplexes that proved to efficiently silence the genes of interest in virtually any cell line. The duplexes are stable at ambient temperatures and are delivered to the cells by "passive transport" independently of viral virus- or lipid-mediated transfection reagents. Therefore, sdRNA have generally no toxicity for the cells.

The duplexes are prepared from separately synthesized single strands and routinely characterized structurally and functionally. We re-estimate the concentration by measuring the OD and check the duplex formation by Native 15%, 20% or 4-20% TBE gel electrophoresis (see the figure below). The sdRNA duplexes efficacy is routinely tested and validated in cell culture to induce 60-70% degradation of the target mRNA (by qRT-PCR).

PROPERTIES

The sdRNA reagents are supplied as 0.1 – 1 mM solutions in sterile RNase-, DNase-, endotoxin-free water (≤0.025 EU/ml).

Our oligonucleotides are purified by either standard desalting or by High Performance Liquid Chromatography (HPLC). Desalting is acceptable for many *in vitro* applications, including functional screenings and target validation experiments. For *in vivo* applications, large-scale synthesis or fluorescently tagged sdRNA's we usually recommend HPLC purification.

Stock concentration: 100 µM - 1 mM
Working concentration: 100 nM - 2 µM
Storage temperature: -20ºC - -80ºC
! Do not store stock dilutions !

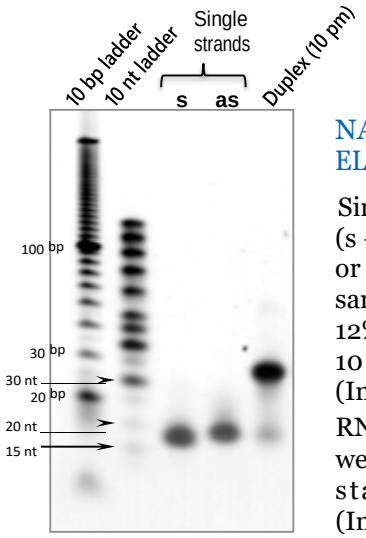
STORAGE AND HANDLING

For long-term storage, keep sdRNA solutions in -20˚C to -80˚C. The sdRNA reagents are stable at ambient temperatures and in a few freeze-thaw cycles, however, we do recommend to prepare stock aliquots. For short-term experiments (1 – 2 weeks), we advise to keep sdRNA duplexes at 4˚C.

Preparing sdRNA for transfection, it is important to make sure that there are no precipitates in the solution. Therefore**, before each experiment bring the duplexes to RT (or thaw the reagents completely), warm them up at 37˚C for 5 min, thoroughly vortex and briefly spin down.**

APPLICATION

For use in tissue culture, sdRNA is generally diluted to 0.1 – 2 µM with common tissue culture medium (EMEM, RPMI, etc). Reducing FBS (or other serum supplements) in the transfection medium significantly increases the efficacy of sdRNA. Optionally, sdRNA can be applied on cells for 24 – 48 h in reduced serum medium before restoring serum to the desired concentration.



NATIVE TBE GEL ELECTROPHORESIS

Single stranded oligonucleo-tides (s – for sense; as – for antisense) or a duplex were mixed with TBE sample buffer and loaded into 4-12% TBE polyacrylamide gel at 10 pmole/well. 10 bp DNA ladder (Invitrogen, 10821015) and 10 nt RNA marker (Affymetrix, 76410) were used as references. Gel was stained with SYBR Gold (Invitrogen, S-11494).