raPOOL FAQ

What is the difference between raPOOLs and other commercial biotinylated probes?

Other commercial suppliers typically offer biotinylated probes individually. raPOOLs in contrast, are sold as a highly complex mixture of 30 single-stranded biotinylated DNA probes. The high complexity of raPOOLs increases chances of association with the target RNA, making RNA pulldown more robust and reliable.

How are raPOOLs designed?

The 30 probes within a raPOOL are tiled across the target RNA of interest. siTOOL's design algorithms additionally incorporate off-target filters to minimize non-specific binding, and optimized thermodynamics to increase probe binding affinity. Custom specifications such as targeting selected isoforms or special species can also be incorporated on request.

What are the characteristics of the biotin attachment to raPOOL probes?

The biotin is at the 3' end of the single-stranded DNA probes that make up the raPOOL. The biotin is linked with an 11 atom spacer arm to avoid stearic hindrance.

What proteins/nucleic acids are known to interact with MALAT1, the positive control raPOOL?

The MALAT1 raPOOL was noted by customers to successfully pull down published proteins that include SR splicing factors (SRSF 2, SRSF3, SRSF5) and heterogeneous nuclear ribonucleoproteins (HNRNPC, HNRNPPF). Publication of MALAT1 interaction with SRSFs:

Tripathi, V et al. (2010) The Nuclear-Retained Noncoding RNA MALAT1 Regulates Alternative Splicing by Modulating SR Splicing Factor Phosphorylation. Mol. Cell. 39, 925–938

The MALAT1 raPOOL was also noted by customers to enrich published DNA targets HEXIM1, RNF40, PNN and PS2 in PC-3 cells. Publication of MALAT1 interaction with chromatin targets:

West, J. A et al. (2014) The long noncoding RNAs NEAT1 and MALAT1 bind active chromatin sites. Mol. Cell. 55, 791–802

Is it necessary to perform sonication prior to the RNA pulldown?

Sonication is performed to thoroughly disrupt cells and randomly shear nucleic acids to increase efficiency of their pulldown, hence improving detection sensitivity. It is recommended and sonication conditions have to be optimized depending on the number and cell type used.

Is it necessary to perform cross-linking prior to the RNA pulldown?

Cross-linking is performed to stabilize molecular interactions before the pulldown. It may not always be necessary and depends largely on the RNA of interest as well as the goal of the pulldown (i.e. characterization of DNA vs. protein interactors). Different forms of cross-linking such as formaldehyde, glutaraldehyde and UV can be used.

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What are the key factors determining RNA pulldown efficiency?

In terms of importance, starting material is key. Sufficient amounts of RNA need to be enriched to detect proteins. Hence, target RNAs that are lowly abundant may require the analysis of large numbers of cells which can pose challenges in terms of cell culture storage and handling capacity. Other notable factors that influence enrichment efficiency include cross-linking and sonication conditions, salt concentrations in the buffer, hybridization time (overnight hybridization has been reported to vastly improve RNA enrichment), and probe concentrations. Useful blogpost: Tips for optimizing RNA affinity purification

How are raPOOLs shipped? Are they stable at room temperature?

raPOOLs are shipped freeze-dried in powder form. This makes them stable at room temperature until resuspension in nuclease-free water, which is provided. raPOOLs have been tested to be stable for at least two weeks at room temperature and at least a day at 50°C, hence shipment delays in warm climates should not adversely affect raPOOL activity.

How many pulldown experiments can I perform with 10nmol raPOOL?

This is dependent on the target RNA of interest. With the recommended starting amount of 100pmol of raPOOL per ml of lysate, 10 nmol should be sufficient for at least 90 pulldown experiments.

Can raPOOLs work for mRNA?

Yes, the same principle of RNA enrichment with raPOOLs applies as well to messenger RNA.

What is the shelf-life of a raPOOL?

raPOOLs are stable for at least 6 months when stored at -20°C in nuclease-free water. Splitting up larger volumes into multiple aliquots is strongly recommended to avoid multiple freeze thaw cycles.

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