Hybridization Buffer to Simplify Quantitation of SiRNA-Conjugated Antibody

Ivy Fine Chemicals, Catalog No. **HY-1301.** 5 mL for 100 Hybridizations
Stable at 4°C for two years. Laboratory Use Only

Product Description and Features:

Quantitation of both SiRNA and antibody are important for the research and development of SiRNA-Conjugated Antibody products. More important are the quantitative correlation and specificity from SiRNA and antibody within a single molecule.

While SiRNA-Conjugated Antibody products have advanced fast into clinical development, the analysis and quantitation of SiRNA-Conjugated Antibody products from clinical setting to manufacturing environment are challenging and left behind.

Here we have developed a unique hybridization buffer to simplify and enhance the hybridization of sequence-specific biotinylated oligo DNA with the specific SiRNA conjugated on the antibody. This hybridization buffer maintains the sample solubility during denaturing heating without an issue of protein aggregation. Once the specific SiRNA is captured through hybridization on streptavidin surface, you can quantitate the antibody scaffold via classic ELISA (e.g., HRP conjugated anti-human IgG detection antibody). Our unique approach and design allow you to analyze both SiRNA and antibody scaffold in a single assay for clinical and development samples and understand the correlation of SiRNA with antibody scaffold without using time consuming sample purification and complex mass spec technology.

Procedures

❑ Warm up the buffer at a warm water bath before use.

❑ Hybridization Setup in 96-well PCR Plate: add and mix 40 µL of SiRNA-Conjugated Antibody, 5 µL of biotinylated oligo DNA specific to SiRNA sequence (e.g., complement to conjugated SiRNA sense strand), and 45 µL of Hybridization buffer. The final dilution of SiRNA-Conjugated Antibody needs to be tested, often with an initial starting concentration at a few hundred µg/mL. The oligo DNA concentration will be 2-3 folds of SiRNA-Conjugated Antibody in molar ratio. Make sure no reducing chemicals (e.g., DTT) is present in the hybridization setup.

❑ Hybridization: set up PCR at 95°C for 10 minutes, followed by hybridization at room temperature for 30 minutes

❑ Quick spin of the PCR plate and transfer 80 µL of hybridization to a streptavidin-coated plate. Gentle shaking at room temperature for 30 minutes, followed by three to four washes in PBS buffer containing 0.01% Tween 20.

❑ Add 100 µL of properly diluted HRP-conjugated anti-human IgG detection antibody prepared in standard BSA or casein blocking buffer for ELISA and incubate at room temperature with gentle shaking for 60 minutes. If your antibody scaffold is not a whole IgG, use the specific antibody as your detection such as Fab or VHH.

❑ Wash the plate three to four times in PBS containing 0.01% Tween 20, followed by colorimetric development in TMB substrate.

❑ The binding results relative to Reference Standard represent the integrity of SiRNA conjugation to Antibody. Such a hybridization and binding testing can provide a quality control of SiRNA-Conjugated Antibody products and a pharmacokinetics of clinical samples.

Ivy Fine Chemicals
1879 Old Cuthbert Road, Suite 23, Cherry Hill, NJ 08034, USA
Phone: 856-465-8550, Fax: 856-616-1161
Email: sales@ivychem.com; support@ivychem.com
Web: http://www.ivychem.com