Question: What is the recommended protocol to test DFHBI and DFHBI-1T fluorescence?

Answer: For all our DFHBI and DFHBI-1T batches, we verified fluorescence with just Spinach2™ aptamer alone in vitro. The protocol is as follows:

1. PCR Spinach2™ sequence with T7 binding site. Gel-purify the 114 bp product.

Spinach2™ sequence:
GATGTAACTGAATGAAATGGTGAAGGACGGGTCCAGTAGGCTGCTTCGGCAGCCTACTTGT
TGAGTAGAGTGTGAGCTCCGTAACTAGTTACATC

T7-Spinach2™ 5': taatacgactcactatagg GATGTAACTGAATGAAATGGTGAAGGACG
Spinach2™ 3: GATGTAACTAGTTACGGAGCTCACACTC
(Anneals at 55°C)

2. Assemble in vitro transcription reaction using MEGAsciript™ T7 kit (Ambion™) per manufacturer protocol with 100 ng PCR template. Incubate in 42°C water bath overnight.

3. Treat with 1 uL DNase for 15 min at 37°C. Purify by phenol:chloroform extraction and isopropanolol precipitation (as described in the MEGAsciript™ T7 kit manual). Resuspend RNA in water.

4. Make 5X aptamer buffer: 0.75 M KCl, 200 mM HEPES, and 0.5 mM MgCl2

5. Dilute 1 μM RNA in 1X aptamer buffer with or without 20 μM DFHBI. In a spectrofluorimeter, excite the solution at 447 nm (for DFHBI) and 482 nm (for DFHBI-1T) and measure fluorescence emission from 495-600 nm.