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

Answer: For all our DFHBI and DFHBI-1T batches, we can verify fluorescence with Spinach2™ or Broccoli™ aptamer alone *in vitro*. The protocol is as follows:

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

Spinach2™ sequence:
GATGTAACTGAATGAAATGGTGAAGGACGGGTCCAGTAGGCTGCTTCGGCAGCCTACTTGTTGA
GTAGAGTGTGAGCTCCGTAACTAGTTACATC

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

1b. PCR Broccoli™ sequence with T7 binding site. Gel-purify the 49 bp product.

Broccoli™ sequence:
GAGACGGTCGGGTCCAGATATTCGTATCTGTCGAGTAGAGTGTGGGCTC

T7-Broccoli™ 5': taatacgactcactatagg GAGCCCACACTCTACTCG
Broccoli™ 3: GAGCCCACACTCTACTCG
(Anneals at 50°C)

2. Assemble *in vitro* transcription reaction using any commercial T7 *in vitro* transcription kit per manufacturer protocol with 100 ng PCR template. Incubate in 42°C water bath overnight.

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

Alternately, Bio-Rad Micro Bio-spin P30 gel columns can be used to purify and concentrate RNA.

4. Make 5X aptamer buffer: 0.75 M KCl, 200 mM HEPES, and 0.5 mM $MgCl_2$

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.