AptaBeacon™ (Fluorescent-Quencher) Demonstration Kit

Kit Contents (For Guaranteed 20 Reactions)

Tube Number	Component	Amount	Storage Conditions
1	Adenosine AptaBeacon (fDNA)	1.3 nmols in 13 uL (100 uM)	Keep Frozen
2	Adenosine AptaQuencher (qDNA)	2.3 nmols in 23 uL (100uM)	Keep Frozen
3	Salt Buffer 1	250 μL	Keep at 4°C
4	Salt Buffer 2	250 μL	Keep at 4°C
5	Adenosine (ADE) Solution	250 μL (5 mM)	Keep at 4°C

Composition of Materials

- Adenosine AptaBeacon Sequence: 5'-ACC TGG GGG AGT ATT GCG GAG GAA GGT /36-FAM/-3'
- Adenosine AptaQuencher Sequence: 5'-/5IABkFQ/ ACC TTC CTC CG-3'
- Salt Buffer 1: 100 mM NaCl and 4 mM $MgCl_2$.
- Salt Buffer 2: 152.6 mM NaCl, 0.84 mM $MgCl_2$, 5.26 mM KCl, and 21.1 mM Tris.
- Adenosine Solution: 5 mM Adenosine.

Background Information

Aptamers are DNA or RNA oligonucleotides that bind to a target with high affinity and specificity. Typically, they range in size between 20-100 base pairs. Aptamers are developed through an *in vitro* process called SELEX (Systematic Evolution of Ligands by Exponential Enrichment). Once developed, aptamers can be used for analytical, diagnostic, or therapeutic purposes. One application for aptamers is to use them as molecular beacons, which are oligonucleotides that detect targets by a fluorescence signal.

This AptaBeacon Kit is designed with a two-strand system, in which there is a fluorescently labeled Adenosine aptamer (the AptaBeacon) and a complementary quenching strand (the AptaQuencher). When these two strands are mixed in solution, the AptaBeacon and AptaQuencher will hybridize, resulting in a decreased fluorescent signal. When the target, Adenosine, is added, the fluorescently tagged AptaBeacon will preferentially bind to the target and release the AptaQuencher in the process. This conformation change causes physical separation of the fluorophore and quencher and will result in an increased fluorescent signal.

The Adenosine AptaBeacon (fDNA) in this kit is labeled with FAM on the 3' end and the AptaQuencher strand (qDNA) is modified with an Iowa Black quencher on the 5' end. When these two modifications are in close proximity, decreased fluorescence will be observed as a result of the fluorescence-quenching Iowa Black modification. If these two modifications are separated, as they are when the Aptamer binds to the target (Adenosine), increased fluorescence will be observed.

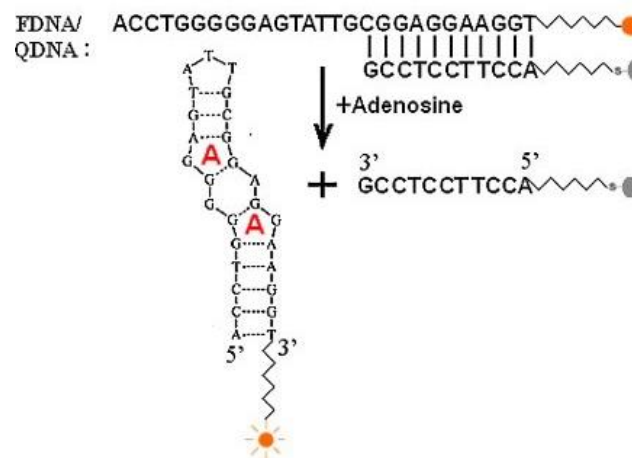

Figure 1. Schematic Representation of AptaBeacon Function.[1] The AptaBeacon ("FDNA") and AptaQuencher ("QDNA") are modified with a fluorescent FAM label and an Iowa Black quencher, respectively. These two strands will remain hybridized until Adenosine (the target) is added. Incubation with the target will result in the release of the AptaQuencher and a corresponding increase in the fluorescent signal.

Kit Instructions

1. **Thaw the fDNA and qDNA.** The fDNA is provided at 100 μM concentration in 5X Salt Buffer 1. The qDNA 100 μM concentration in nuclease-free water. (Store stock solutions in freezer).
2. **Create the fDNA-qDNA Duplex.** Mix the fDNA and qDNA in a 1:4 volume ratio (fDNA:qDNA), with 5 μL of fDNA and 20 μL of qDNA. Heat the mixture for 1 min. at 90°C, 5 min. at 60°C, and 5 min. at 25°C. The Duplex concentration is 20 μM, as it is equal to that of the fDNA. (Store at 4°C).
3. **Make 1 μM fDNA Solution.** First, mix 5 μL of 100 μM fDNA with 20 μL of nuclease-free water, creating a 20 μM fDNA solution. Then, mix 2 μL of 20 μM fDNA with 38 μL of Salt Buffer 2. The fDNA concentration is now 1 μM. (Store at 4°C).
4. **Make 1 μM Duplex Solution.** Mix 2 μL of 20 μM Duplex with 38 μL of Salt Buffer 2. The Duplex concentration is now 1 μM. (Store at 4°C).

IMPORTANT: The following measurements should be done in triplicates, so each of the "tubes" in steps 5-7 should be made three times, for a total of nine tubes in all.

5. **Make fDNA-only (Positive Control) Tube.** Mix 5 μL of 1 μM fDNA with 5 μL of DEPC. (Make three of these).
6. **Make Duplex-only (Negative Control) Tube.** Mix 5 μL of 1 μM Duplex with 5 μL of DEPC. (Make three of these).
7. **Make Duplex-ADE Tube.** Mix 5 μL of 1 μM Duplex with 1 μL of DEPC and 4 μL of 5 mM ADE. (Make three of these).
8. **Incubate the tubes from steps 5-7 at 45°C for 5 min.** Allow these to cool to room temperature naturally.
9. **Make Salt Blank.** First, mix 2 μL of Salt Buffer 1 with 38 μL of Salt Buffer 2. Then, take 5 μL of that salt solution and mix with 5 μL of DEPC. This is the Salt Blank. (Store at 4°C).
10. **Measure fluorescence of each tube using a fluorometer on the FAM setting.** Be certain to blank the instrument with the Salt Blank from step 9.
11. **The data can be imported to Excel and graphed to provide a better visual of the AptaBeacon's function.** Sample data and graphs are included on the back of this sheet for further explanation.

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Sample Name	RFU 1	RFU 2	RFU 3	Avg.	Std. Dev.
fDNA only	6304	6461	6239	6335	93
Duplex only	4592	4637	4543	4591	38
Duplex + 0.5 mM ADE	5590	5584	5632	5602	21
Duplex + 1 mM ADE	5982	5992	6016	5997	14
Duplex + 1.5 mM ADE	6310	6463	6464	6412	72
Duplex + 2 mM ADE	6387	6624	6701	6571	133

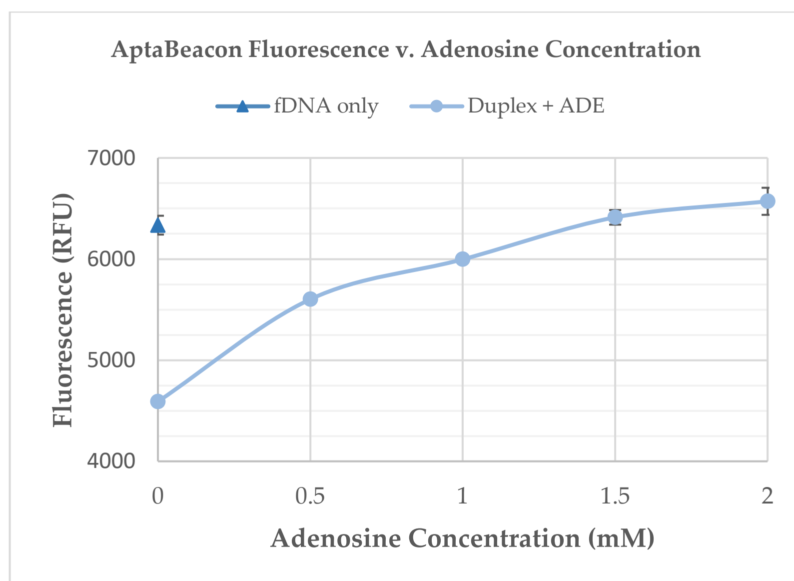

Figure 2. AptaBeacon Fluorescence as a Function of Adenosine Concentration.[2] The fDNA by itself (dark blue triangle) provides a positive control value for the maximum fluorescence of the solutions, as fDNA and Duplex concentration are equal. The existence of the Duplex results in reduced fluorescence (as seen at zero Adenosine concentration) while the presence of Adenosine increases the fluorescent signal (as seen when Adenosine concentration increases). When the target molecule, Adenosine, is added to the solution, the fDNA-qDNA Duplex dissociates as the fDNA strand binds to the target. This pulls the quencher (on the qDNA) away from the fluorophore (on the fDNA) and causes an increased fluorescent signal, as the physical distance between strands means that the quencher can no longer prevent fluorescence of the fluorophore. All data included in this graph is listed in the table above. Notice that the fluorescence of each sample was measured in triplicates, as three tubes of each sample were made.

Sample Name	RFU 1	RFU 2	RFU 3	Avg.	Std. Dev.
fDNA only	6304	6461	6239	6335	93
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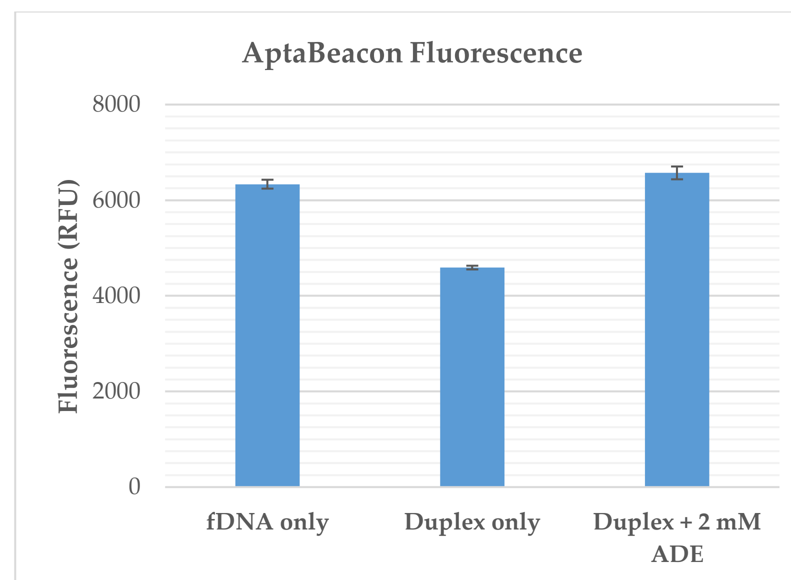

Figure 3. AptaBeacon Kit Fluorescence.[2] This data represents that which should be observed with this kit. The first column, “fDNA only,” provides the maximum expected fluorescence for the experiment (positive control), since none of the fluorophore is quenched in this solution. The middle column, “Duplex only,” provides the minimum expected fluorescence for the experiment (negative control), since the Duplex results in fluorescence quenching by the close proximity of the fDNA and qDNA strands. The last column, “Duplex + 2 mM ADE,” shows the effect of adding the target molecule; the fDNA releases from the Duplex and binds to the Adenosine, leaving the qDNA strand unable to quench the fluorophore. All data included in this graph is listed in the table above. Notice that the fluorescence of each sample was measured in triplicates, as three tubes of each sample were made.

References

[1] Zhang, J.; Wang, Y.; Xue, J.; He, Y.; Yang, H.; Liang, J.; Shi, L.; Xiao, X. A Gold Nanoparticles-Modified Aptamer Beacon for Urinary Adenosine Detection Based on Structure-Switching/Fluorescence-“Turning On” Mechanism. J. Pharmaceut. Biomed. **2012**, *70*, 362-368.

[2] Graphs were made with MS Excel.

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