Abstract: We present a microfluidic aptamer-based biosensor for specific cocaine detection. The device consists primarily of a microchamber packed with aptamer-functionalized microbeads that act as a sensing surface, integrated with an on-chip heater and temperature sensor. The sensor employs a fluorescence resonance energy transfer (FRET), system in which a fluorophorequencher pair of Carboxyfluorescein and Dabcyl generates a signal-on response to cocaine. We demonstrate device operation by successfully detecting cocaine with a four orders of magnitude linear response in micromolar to nanomolar concentrations. The detection limit of the device is further lowered to 10 pM by concentration of a highly diluted cocaine sample, which compares well with the most sensitive detection techniques currently available. The temperature-dependent binding of aptamer-analyte complexes is then used to effect thermal release of cocaine from the sensing surface. It is found that a temperature of 37°C can fully regenerate the sensor in pure buffer. Furthermore, testing indicated that sensor response is consistent after repeated regeneration. These results demonstrate that aptamer-based sensing on a microfluidic platform has the potential to enable low-cost, rapid, and highly specific detection of cocaine in practical applications.