

**Appendix 4: Preparation of the Positive Control Target Working Solution (1 of 1).**

Hydrate and dilute the Positive Control gBlock (HMgBlock135m) in TE pH 7.5 dilution buffer according to instructions below to obtain the working concentration of 5E2 copies/ $\mu$ L. Store dilutions at -20 degrees Celsius. The working solution can be stored at -20 or 4 degrees C. A fresh working solution should be prepared from the frozen 5E3 dilution every 90 days.

<b>HMgBlock135m Positive Control: Hydration and Dilution Procedure</b>	<b>Concentration (copies/<math>\mu</math>L)</b>
Centrifuge the lyophilized gBlock (1000 ngrams) prior to opening. Hydrate in original tube with 487.5 $\mu$ L TE dilution buffer. Vortex briefly and incubate at 50 degrees C for 20 minutes. Vortex briefly and centrifuge again to bring liquid contents to the bottom of the tube.	2E9
Mix 10 $\mu$ L of the 2E9 stock + 990 $\mu$ L TE in a new tube. Centrifuge to bring liquid contents to the bottom of the tube.	2E7
Mix 10 $\mu$ L of the 2E7 dilution + 990 $\mu$ L TE in a new tube. Centrifuge to bring liquid contents to the bottom of the tube.	2E5
Mix 25 $\mu$ L of the 2E5 dilution + 975 $\mu$ L TE in a new tube. Centrifuge to bring liquid contents to the bottom of the tube.	5E3
Mix 50 $\mu$ L of the 5E3 dilution + 450 $\mu$ L TE in a new tube. Centrifuge to bring liquid contents to the bottom of the tube.	5E2