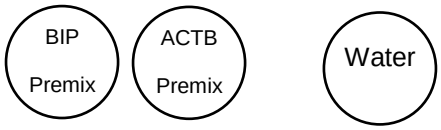
Pre-Test 1
Check amplification and Ct value (1st PCR)

1 SET UP AND POINT OF ATTENTION

Label three 1.5mL tubes as below.



Aliquot 1mL of pure water in the labelled tube from provided 50mL tube. Use this water exclusively during the entire experiment in order to prevent contamination of the stock water.

Always use filtered tip for pipetting.

Dispose used tip immediately and always use new filtered tip for liquid/reagent handling in order to avoid any kind of contamination.

2 REACTION MIX PREPARATION

Add followings in labelled 1.5mL tubes (one tube for one primer set; 2 tubes in total).

ddH_2O	16	
2x Master Mix	20	
50x ROX	0.8	
10μM each Primer mix.	1.2	(BiP or ACTB) (μL)
Total	38	

After adding all, mix by vortexing and spin down the tubes.

Aliquot 19μL each in PCR tubes (provided 2-well strips).

Make a label on the upper side of the tube to distinguish samples and your group (make a small labeling; big label affects the signals on detection).

Add 1μL of reverse transcribed cDNA.

Each tube contains primers and cDNAs as below.

BiP primer SampleA cDNA	BiP primer SampleB cDNA	ACTB primer SampleA cDNA	ACTB primer SampleB cDNA

Mix by vortexing and spin down the tubes.

Apply to the real-time PCR equipment (StepOne Plus) and start reaction.

（If you do not start reaction immediately, keep the tubes in dark and at 4℃ in a refrigerator.）

Important: For real-time PCR, do not use colored pen and avoid big labeling because the ink would interfere the signal detection. You have to avoid labeling on top of the tube/well because the light signal should pass though the lid.