DATE:_____

PREPARED BY:_____

SALMONELLA BAX SAMPLE PREP FOR ENRICHMENTS

☐ Label whirlpak bags with sample numbers

Sample#	Sample Description	Sample#	Sample Description	Sample#	Sample Description	Sample#	Sample Description	Sample#	Sample Description

☐ Weigh out 24 grams of pet food in a weigh boat and place in filter whirlpak bag. Actual weight in grams_____

☐ Add 1g inoculated kibble

Add 225 ml of lactose broth to the pet food and incubate 1 hour at RT. TIME IN_____TIME OUT_____

☐ Measure pH and adjust as needed with sterile NaOH to pH of 6.8

☐ Place bags in stomacher and stomach for 1 minute at 230 rpm.

☐ Incubate 24 hours @ 35C. TIME IN_____TIME OUT_____

SAMPLE PREP:

☐Turn on the 37C and 95C heat blocks

☐Place cooling block at 4C

☐Disinfect the capping/de-capping tools with 70% ETOH.

☐Prepare lysis buffer by adding 150 ul of protease to 12 mL of buffer. Place on ice.

☐ Label lysis tubes. Use no less than 4 tubes tubes from the 8 tube strip. Be sure to have an empty tube at the end.

☐Aliquot 200 ul of lysis buffer to _____tubes.

☐Aliquot 5ul of the enrichment into the lysis buffer. Do not run the sample down the side of the tube, add directly to the buffer.

☐Cap tubes as a strip is completed using the capping/de-capping tool.

☐Confirm temperature of 37C heat block. **Temp reading**_____

☐Place the prepared lysates on the 37C block and incubate for 20 minutes. **Time in_____Time out_____**

☐Confirm the temperature of the 95C heat block. **Temp. reading_____**

☐Remove the lysates from the 37C block, place in 95C block and heat for 10 minutes.

Time in_____Time out_____

☐Remove the lysates from the 95C block and place in cooling block.

☐Incubate for at least 5 minutes. **Time in_____Time out_____**

If preparing BAX assay, move to next section.

If assay is to be run at a later date, place the lysates in a sample rack, date/initial the plate, and store the lysates at 4C up to 1 week, or at -20C up to 2 weeks.

Place primary enrichment in cold room.

> ****** NOTE:*** *FDA BAM permits holding primary enrichments (enrichment containing original 25g sample) to be held at 4°C for up to 48 h to allow for additional testing, if necessary. Results taken from enrichments beyond 48 h should not be considered in analysis.**********

BAX PREP:

☐Label tubes with sample numbers

☐Prepare sample plate map.

☐Enter sample IDs, save the plate map and launch BAX program

(Always do this step prior to preparing the samples, the instrument needs time to reach temperature).

Remove appropriate number of sample strips from the kit and place them in the cooling block.

(Note: The strips containing the reagent pellet must be kept on the cold block and not held at RT for more than 30 minutes).

- Carefully remove the caps using the de-capping tool. Discard caps and replace with new caps when finished.

☐Hydrate the reagent pellet with 50ul of lysate. Pipet the sample down the side of the tube.

☐Re-cap the assay tubes with the visual caps using the capping tool.

- Place the samples on the instrument
- Select “Run Full Process”
- Don’t leave the instrument unattended until it shows that it is performing PCR.

CONFIRMATION OF NEGATIVE BAX RESULTS:

☐ Inoculate 10ml TT with 1ml and 10ml RV with 0.10ml of primary enrichment.

☐ Incubate TT at 35C for 24 +/- 2 hours. Time in_____Time out_____Temp_____

☐ Incubate RV at 42C for 24 +/- 2 hours. Time in_____Time out_____Temp._____

☐ Spread plate 10ul of TT and RV enrichments on XLD and HE.

☐ Incubate plates at 35C for 24 +/- 2 hours. Time in_____Time out_____Temp._____