ExoSAP-IT Sample Cleanup		
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EXOSAP-IT Sample Cleanup

RETIRED

1 Purpose:

1.1 Prior to cycle sequencing, unincorporated primers and nucleotides present in the amplification reaction are deactivated by the addition of ExoSAP-IT.

1.2 It is very important for these sample names to be in 3130xl format; do not use spaces or the following characters: \ / : * " > < | ? ')

1.3 In LIMS, Navigate to the test batch's *Output Sample* [Data Entry] page.

1.3.1 The "Vol, Misc" must be entered by the analyst, and then the batch should be saved. This will trigger the automatic values based on the samples' previous runs to populate in the data.

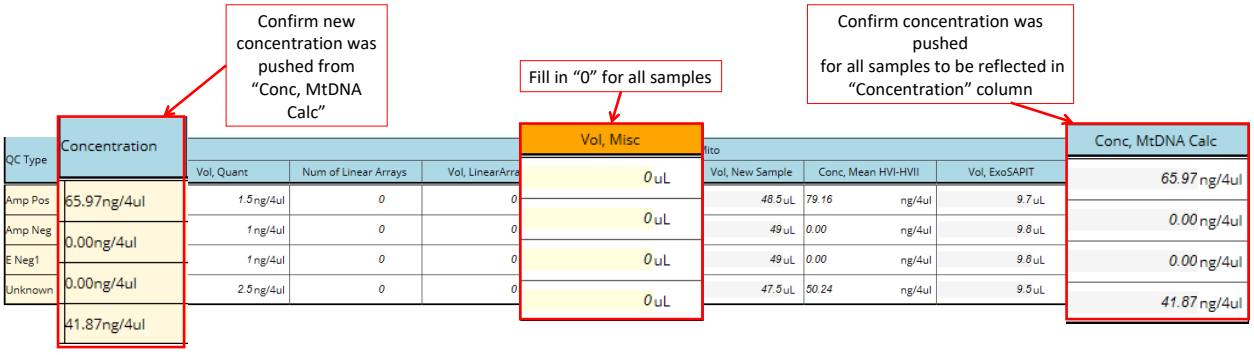
1.3.1.1 Fill in the *Vol, Misc* column as "0" for all samples.

1.3.2 The calculations for the ExoSAP-It volume and the new concentrations will automatically execute.

1.4 There should be 1ul of ExoSAP-IT added for every 5ul of sample in the amplification tube.

1.5 Select the values in the "Conc, MtDNA Calc" column and **Push Concentration.**

1.6 The test batch set up and Batch Setup Review are usually performed by the analyst who had previously reviewed the Agilent run(s) for the same samples. Analyst must verify that the values present in the "Conc, MtDNA Calc" column match those in the "Concentration" column after pushing concentration.



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2 ExoSap-It

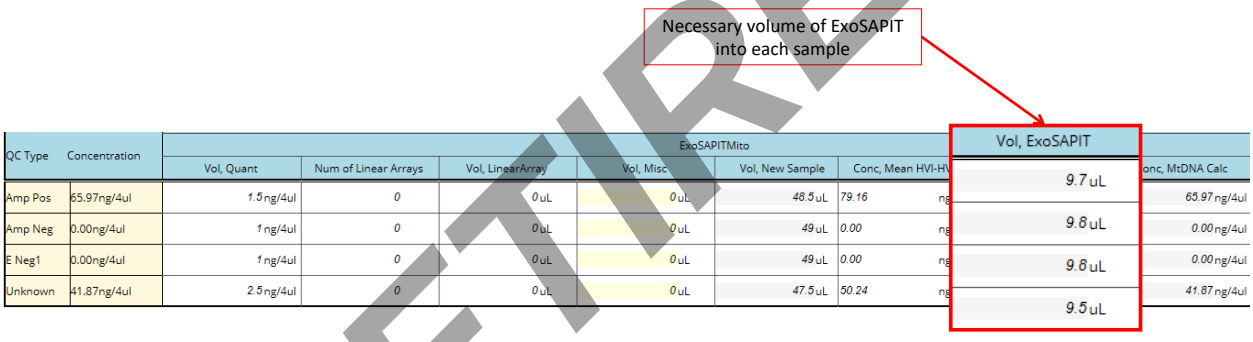
2.1 Confirm the tube label and sample description for each sample. Every run should include positive control and an amplification negative control.

2.2 Fill in the Performed By tab in LIMS. Update the batch description with the instrument name that you are planning to use, the year, and the run number for that year.

2.2.1 Use "S" for Spiderman and "B" for Batman (Ex. S20-045)

2.3 Retrieve ExoSAPIT reagent and fill in lot number in LIMS.

2.4 Referring to the "Vol, ExoSAPIT" column on the Data Entry page, add the necessary volume of ExoSap-IT to each of your sample tubes.



QC Type	Concentration	ExoSAPITMito								onc, MtDNA Calc
		Vol, Quant	Num of Linear Arrays	Vol, LinearArray	Vol, Misc	Vol, New Sample	Conc, Mean HVI-HV		Vol, ExoSAPIT	
Amp Pos	65.97ng/4ul	1.5 ng/4ul	0	0 uL	0 uL	48.5 uL	79.16	ng	9.7 uL	65.97 ng/4ul
Amp Neg	0.00ng/4ul	1 ng/4ul	0	0 uL	0 uL	49 uL	0.00	ng	9.8 uL	0.00 ng/4ul
E Neg1	0.00ng/4ul	1 ng/4ul	0	0 uL	0 uL	49 uL	0.00	ng	9.8 uL	0.00 ng/4ul
Unknown	41.87ng/4ul	2.5 ng/4ul	0	0 uL	0 uL	47.5 uL	50.24	ng	9.5 uL	41.87 ng/4ul

2.5 Place the sample tubes in the thermal cycler and use the following settings to incubate the samples:

9700 Thermal Cycler User: mtDNA File: exosap-it	The ExoSAP-IT file is as follows: - Soak at 37°C for 15 minutes - Soak at 80°C for 15 minutes Storage soak at 4°C indefinitely

2.6 Start the run by performing the following steps:

2.6.1 The main menu options are RUN CREATE EDIT UTIL USER. To select an option, press the F key directly under that menu option.

2.6.2 Verify that the user is set to "mtDNA." If not, select the USER option (F5) to display the "Select User Name" screen.

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2.6.3 Use the circular arrow pad to highlight "mtDNA." Select the ACCEPT option (F1).

2.6.4 Select the "exosap-it" file, and press the RUN button (F1).

2.6.5 Verify that the reaction volume is set to 50 µL and the ramp speed is set to **9600 (very important)**.

2.6.6 If all is correct, select the START option (F1).

2.7 The run will start when the heated cover has sufficiently pre-heated (>100ºC). The screen will then display a flow chart of the run conditions. A flashing line indicates the step being performed; the hold time is counted down. Cycle number is indicated at the top of the screen, counting up.

2.8 Record the instrument usage in LIMS. Fill out the program as "Exosapit."

2.9 Upon completion of the amplification, remove samples and press the STOP button repeatedly until the "End of Run" screen is displayed. Select the EXIT option (F5). Wipe any condensation from the heat block with a lint free wipe and pull the lid closed to prevent dust from collecting on the head block. Turn the instrument off.

2.10 From this point forward, the ExoSAP-IT treated DNA sample will be the source of DNA template for all further mtDNA sequencing reactions.

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