Troubleshooting Guide for PERFORMA® DTR V3 96-Well Short Plates

PROBLEM	POSSIBLE CAUSE	SUGGESTED SOLUTIONS
Signal Intensity low	Less than 10 µl of sequencing reaction loaded	Adjust the sequencing reactions to 10µl before loading on the plate
	Centrifuge conditions were not optimal	• Recalculate rpm to achieve 850 x g for your centrifuge • Change pre-spin time to 3 min at 750 x g or decrease pre-spin time to 2-2.5 min at 850 x g • Centrifuge samples for 5 min at 850 x g • If adjustable, use fast acceleration centrifugation mode
	Sequencing reaction conditions were not optimal	Adjust sequencing reaction parameters; consult sequencing reagent manufacturer's recommendations
	Sample volume too low for sequencer	Add deionized water or sample buffer to purified reaction before loading on the sequencing instrument
Dye blobs	Too large a volume was loaded	• Load no more than 15 µl • If using > 3 µl BigDye® v 3.1 per 15 µl reaction, consider loading only 10 µl on the Performa® DTR V3 96-Well Short Plate • Consider using Performa® DTR Standard Plate for sample volumes > 15 µl, or sequencing reactions containing more than 3 µl BigDye®
	The concentration of BigDye® v. 3.1 was too high (*i.e.* More than 3 µl BigDye® v 3.1 per 15 µl reaction was loaded)	If the sequencing reaction conditions cannot be altered, consider loading only a portion of the reaction for cleanup and sequencing.

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Dye blobs *cont…*	Centrifuge conditions were not optimal	• Be certain that the pre-spin is for 3 min at 850 x g • Recalculate rpm to achieve 850 x g for your centrifuge conditions • Decrease sample spin to 3 minutes at 850 x g
	Samples were not loaded properly	Load the samples slowly, drop-wise to the center of the gel matrix
	Signal intensity too high	Apply only a portion of sequencing reaction to the plate; high signal strengths exaggerate the effect of small quantities of dye terminator in the purified reaction
	Sequencing reaction conditions were not optimal	Adjust sequencing reaction parameters; consult sequencing reagent manufacturer's recommendations
Truncation of 5' sequence	Centrifuge conditions were not optimal	• Recalculate rpm to achieve 850 x g for your centrifuge • Change pre-spin time to 3 min at 750 x g or decrease pre-spin time to 2-2.5 min at 850 x g • Centrifuge samples for 5 min at 850 x g
	Dye blobs present	See Dye blobs above
	Less than 10 µl of sequencing reaction loaded	Adjust the sequencing reaction to10µl before loading on the plate
	Sequencing reaction conditions not optimal	Adjust sequencing reaction parameters; consult sequencing reagent manufacturer's recommendations

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Recovery volume higher than expected	Centrifuge conditions were not optimal	• Be certain that the pre-spin is performed for 3 min at 850 x g • Recalculate rpm for your centrifuge conditions
	More than 15 µl of sequencing reaction loaded	Decrease volume of sequencing reaction loaded to 10-15 µl
	Plate was stored incorrectly. If you observe a high recovery volume after the first spin, it is likely the plate has been frozen	• Store sealed plates at 4°C • Do not allow plates to freeze
Recovery volume lower than expected	Centrifuge conditions were not optimal	• Recalculate rpm for your centrifuge conditions • Spin samples for at least 5 min at 850 x g
	Less than 10 µl of sequencing reaction loaded	Increase volume of sequencing reaction to 10 -15 µl
	Plates have not been stored correctly. If you observe a low recovery volume after the first spin, it is likely the plate has been dehydrated	• Store plates at 4°C • Plates sealed in original bag can be left at room temperature for several days but should be stored at 4°C

For additional troubleshooting assistance, please contact Edge BioSystems at:

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