



Store the DNA Size Standard 80 Kit in the dark in a -20°C non frost-free freezer.

Introduction

The DNA Size Standard 80 Kit contains two

reference fragments labeled with the D1 (red) dye for use in sizing SNP fragments in the approximate size range 20 nt to 80 nt. The DNA Size Standard 80 Kit is designed to accommodate a wide range of sizes for multiplexed and poolplexed SNP fragments.

Materials Required

Materials provided by Beckman Coulter, Inc.:

- DNA Size Standard 80 Kit (p/n 608395)
- Separation Gel I (p/n 608010)
- Separation Buffer (p/n 608012)
- DNA Separation Capillary Array, 33-75B (p/n 608087)
- Samples Plate (p/n 609801)
- SLS (p/n 608082)

Sample Preparation for Loading into the instrument

Important: Allow Size Standard 80 to thaw and equilibrate at room temperature for at least 15 minutes prior to use. Spin the tube briefly and mix thoroughly by pipetting up and down 5–10 times to ensure balanced size standard peak heights.

1. Only a small amount of the labeled SNP product is required for running on the instrument. The recommended starting point is $0.5 \ \mu$ L of labeled SNP product. If dilution is required, it is recommended that the SNP products be diluted in SLS. Add the sample to 39 μ L of SLS in the sample plate containing 0.5 μ L of the DNA Size Standard 80 Kit.

Note: The amount of labeled SNP product used will depend upon the efficiency of the reaction. The volumes given above are a recommended starting point. Certain SNP samples may require that more or less sample be added. The presence of salt will also interfere with loading of samples into the capillary. Unless the SNP samples are purified following the labeling reaction, salt from the reaction buffer will be present in the sample. In this case, adding more volume can reduce signal due to salt from the sample being preferentially injected into the capillary and thus reducing the amount of DNA fragments loaded.

- Overlay each of the samples with one drop of light mineral oil (provided with the DNA Size Standard 80 Kit).
- 3. Load the sample plate onto the instrument and start the desired method.

Appendices Appendix A

Sample Prep for Running Pooled Samples

When running pooled samples on the instrument, it may be necessary to adjust the relative amounts of SNP sample and DNA Size Standard 80 Kit used. When more than one SNP sample is used, the amount of DNA Size Standard 80 may need to be increased. This is because the more fragments present in a pooled SNP sample, the greater the competition for injection. With pooled SNP samples there will be more fragments and possibly more salt (from the labeling reaction buffer, etc.) competing for injection into the capillary.

The simplest way to determine the amount of sample required is to run the labeled SNP products individually and then adjust the amounts in the pooled samples to give balanced signals. If an individual SNP sample gives a high signal then the amount loaded can be reduced more than a sample that gives low signal. Keep in mind that all signals will be lower in the pooled SNP sample compared to the individual samples. By purifying the labeled products prior to loading onto the instrument, the salt competition can be reduced.

Appendix B

Multiplex and Poolplex SNP Sample Spacing

The minimum recommended spacing between SNP products and also between SNP products and the SNP Reference fragments is 5-8 bases (more spacing for smaller fragments and less for larger fragments). This means that in order for the software to function properly, all SNP products must migrate between the two reference fragments. Be aware that owing to effects of base composition and sequence, migration times of short fragments can differ significantly from predicted times based on size alone. This inability to predict migration time reliably can lead to the overlap of adjacent fragment signals and complicate automated genotyping. Customers running dense primer sets are recommended to run individual reactions with probes of each allele size to determine if signal overlap will occur, and if necessary, design primers with a greater number of bases between adjacent SNP signals. Alternatively, it may be necessary to analyze products with overlapping signals in separate pools.

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GHS HAZARD CLASSIFICATION

CEQ DNA Size Standard Kit-80

H360	May damage fertility or the unborn child.
P201	Obtain special instructions before use.
P280	Wear protective gloves, protective clothing and eye/face protection.
P308+P313	IF exposed or concerned: Get medical advice/attention.
	Formamide>90%

SDS Safety Data Sheet is available at techdocs. beckmancoulter.com

DANGER

EUROPEAN HAZARD CLASSIFICATION

CEQ DNA Size Standard	
Kit-80	T;R61

R61	May cause harm to unbornchild.
S45	In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
S53	Avoid exposure-obtain special instructions before use.

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