MB489_IFU_EN_V2401

Speedy NZY Blue ExoSAP Mix

Catalogue number	Presentations
MB48901	50 rxns
MB48902	500 rxns

Description

Enzymatic cleanup of PCR products stands out for its precision, simplicity, speed, and efficiency compared to alternative methods like spin columns or nucleic acid purification beads. The Speedy NZY Blue ExoSAP Mix features a potent combination of highly specific and pure recombinant Alkaline Phosphatase and Exonuclease activities. These two hydrolytic enzymes are tailored to process PCR products of varying sizes without any loss of sample integrity. Thus, the combinatory activity of this specialized mix eliminates unincorporated primers and nucleotides from PCR reactions, ensuring that resulting amplicons are efficiently processed during DNA sequencing, cloning, genotyping, or any other downstream applications. Speedy NZY Blue ExoSAP Mix was optimized for commonly used PCR buffers, simplifying the cleanup process through the addition in the 1:6 ratio of Enzyme Mix to PCR product. The swift activity lasts only 2 minutes, followed by enzyme inactivation through a brief 1-minute heat exposure at 80°C.

Shipping & Storage Conditions

This product is shipped in dry ice. Upon receipt, store the Speedy NZY Blue ExoSAP Mix immediately at -85°C to -15°C to ensure sustained stability and performance. The product maintains its complete enzymatic activity through up to 10 freeze-thaw cycles. Adhering to these meticulous storage procedures ensures that Speedy NZY Blue ExoSAP Mix will remain stable until the expiry date and deliver reliable and consistent performance in all applications.

Components

COMPONENT	CAT. NO.	TUBES	VOLUME
Speedy NZY Blue ExoSAP Mix	**MB48901**	1	50 µL
	MB48902	1	500 µL

Specifications

Format: One tube – ready to use.

Protocol time: < 5 min.

Compatibility: adaptable for different volumes.

Short inactivation time: 1 min at 80 °C.

Tracking dye

Standard Protocol

The following standard protocol serves as the guideline for the cleanup of PCR products.

1. Remove the Speedy NZY Blue ExoSAP Mix from freezer and keep on ice throughout the procedure.
2. Mix 6 µL of a post-PCR reaction product with 1 µL of Speedy NZY Blue ExoSAP Mix. The reaction volume can be increased as necessary, ensuring that the 1:6 ratio of Mix to PCR product is consistently maintained.
3. Mix thoroughly and quick spin to bring the contents to the bottom of the tube.
4. Incubate the reaction in a thermal cycler or heating block, with a heated lid, with the following protocol:
   a. 37°C for 2 minutes;
   b. 80°C for 1 minute;
   c. Hold at 4°C and transfer samples to ice.
5. The PCR product is now ready for use in DNA sequencing, SNP analysis, or other primer-extension applications. Treated PCR products may be stored at -85°C to -15°C.

Notes: *Before submitting your samples for sequencing determine the amount of your amplicon to ensure that you are submitting the correct amount of DNA depending on the sequencing service. A simple way to do this is to load 5 µL on an agarose gel along with a known amount of a*

control DNA for comparison (use NZYDNA Ladder VIII, MB175). Instead, direct measurement using fluorescent dye-based kit (e.g., Qubit™) will also ensure the proper amount of DNA is submitted.

Quality control assays

Nucleases assay

Speedy NZY Blue ExoSAP Mix is tested for nucleases contamination. To test for DNase contamination, 0.2-0.3 µg of pNZY28 plasmid DNA are incubated with the mix for 14-16 h at 37°C. To test for RNase contamination, 1 µg of RNA is incubated with the enzyme mix for 1 h at 37°C. Following incubation, the nucleic acids are visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the nucleic acids.

Functional assay

Speedy NZY Blue ExoSAP Mix is tested for performance with several PCR buffers.

Troubleshooting

Troubleshooting is often a systematic, meticulous process where varying one parameter at a time and evaluating impacts can unveil the root cause of issues. These adjusted suggestions, incorporating a blend of specificity and exploratory approaches, aim to enhance the clarity and actionability of your troubleshooting guide. Should any other technical or procedural aspects require attention, your feedback and additional information will always be welcomed.

LOW NUMBER OF SEQUENCE READS WITH GOOD ELECTROPHEROGRAM
• **Large amount of sample and or no sufficient enzyme inactivation**
Make sure that your sample complies with the amount/concentration of DNA necessary for sequencing. Make sure you subject the reaction to the heat inactivation after activity.
THERE ARE TWO PEAKS (ONE LARGE AND ONE SMALL) OVERLAPPING IN ONE POSITION OF THE ELECTROPHEROGRAM
• **Low quality of template**
This phenomenon may be explained by one of the two following possibilities. The first is more than one size of PCR product was amplified. If the amplification does not produce a homogeneous product and more than one species is amplified with sequence similarity to another, both can be sequenced with the added sequencing primers. Another explanation may be that unprocessed sample remained on the side of the reaction tube, leading to aberrant reads. Please be sure to vortex and quickly spin the reaction tube after treatment with the Speedy NZY ExoSAP Mix.
NO SEQUENCING SIGNAL
• **DNA concentration is too low**
Peaks are irregular and may appear as if it is just mixed peaks, which are just noise signal. This normally means that the DNA concentration is too low. Primer concentration may also be too low. Check with the sequencing service the required amount of template to send for sequencing and check the concentration by gel electrophoresis or using a fluorescent dye-based kit (e.g., Qubit™) to ensure the proper amount of DNA is submitted.