MB484_IFU_EN_V2301

Lyo Speedy NZY Direct Genotyping Kit

Catalogue number	Presentations
MB48401	100 rxns
MB48402	5 x 100 rxns

Description

The Lyo Speedy NZY Direct Genotyping Kit features a lyophilized formulation of the Speedy Supreme NZYTaq 2x Master Mix, ensuring stable room temperature shipping. The performance of this kit is on par with its liquid-form counterpart, the Speedy NZY Direct Genotyping Kit (MB475). Engineered to process an array of tissue materials, including blood and non-invasive hair samples, these kits promise a seamless, animal-friendly genotyping experience. They integrate a streamlined one-tube sample preparation with a swift PCR amplification, enabling rapid lysis of biological samples, including mouse genotyping tissues samples (tail clipping and ear punch), in under 5 minutes. Lyo Speedy NZY Direct Genotyping Kit is validated for hair sample genotyping, which executed with minimal animal discomfort and without necessitating anaesthesia harmoniously combines ethical and efficient genotyping. The kit includes a potent Speedy Lysis Enzyme Mix, meticulously developed for rapid sample lysis at room temperature. Post-lysis, a brief centrifugation secures the DNA-laden supernatant. Employing an aliquot of this lysate in tandem with the Lyo Speedy Supreme NZYTaq 2x Master Mix ensures lysis and PCR cycling times under one hour (5 min Lysis/Inactivation plus 44 min PCR cycle; total time 49 min). In addition, the master mix reconstitution buffer contains a blue dye for direct loading onto agarose gels, omitting the need for additional dye application. The Lyo Speedy NZY Direct Genotyping Kit also offers an alternative direct PCR protocol, facilitating PCR directly from undiluted, unpurified samples. The process produces ample template for numerous assays, compatible with a 96-well format, and assures sufficient template for a minimum of 20 genotyping PCR reactions. Finally, DNA fragments generated with the Lyo Speedy NZY Direct Genotyping Kit are 3'-dA-tailed and may be cloned into TA cloning vectors, or used for routine downstream analyses or applications, including restriction enzyme digestion, DNA cloning and sequencing.

Shipping & Storage Conditions

Lyo Speedy NZY Direct Genotyping Kit can be shipped from dry ice to room temperature. Upon receipt, store all components of the kit at -85°C to -15°C. Alternatively, Speedy Lysis Buffer and Speedy Lysis Enzyme Mix may be stored at +2°C to +8°C. Correct storage according to these recommendations ensures that the Lyo Speedy NZY Direct Genotyping Kit delivers consistent and reliable results across its lifespan and usage. The kit is robust during shipping, tolerating ambient temperature (+18°C to +25 °C) for up to 1 month. Short-term exposure to temperatures up to 37 °C is permissible for up to 14 days. All components are formulated to be ready to use.

Components

COMPONENT	MB48401 (100 rxns)	MB48402 (5 X 100 rxns)
Speedy Lysis Buffer	5 mL	5 × 5 mL
Speedy Lysis Enzyme Mix	50 µL	5 × 50 µL
Lyo Speedy Supreme NZYTaq 2x Master Mix	2 x for 650 µL	10 × for 650 µL
Speedy Supreme NZYTaq 2x MM Reconstitution Buffer	1500 µL	5 × 1500 µL

Specifications

Expertly formulated for the rapid extraction and amplification of DNA from mouse tissues, the Lyo Speedy NZY Direct Genotyping Kit is also suitable for DNA extraction and amplification from other animal tissues. To ensure reliable animal typing data, it is important to obtain enough DNA in a form suitable to serve as a template for subsequent PCR amplification. The Lyo Speedy NZY Direct Genotyping Kit contains an optimized Speedy Lysis Enzyme Mix and respective Buffer, ensuring proficient DNA release within a 2-minute lysis step. For superior PCR results, we recommend employing an aliquot of this lysate as a PCR typing template. Optionally, the Lyo Speedy NZY Direct Genotyping Kit offers an alternative direct PCR protocol, permitting PCR directly from unpurified and undiluted samples (see below).

Standard Protocol

The following standard protocol serves as a general guideline and a starting point for sample lysis and PCR amplification. Optimal reaction conditions (incubation times and temperatures, concentration of primers and/or template DNA) may vary, although PCR optimization is usually not required. In case you need to fine-tune primer concentrations, test the recommended variations provided in brackets in the table below.

Procedures before starting

- Reconstitute one tube of lyophilized Lyo Speedy Supreme NZYTaq 2x Master Mix by adding 650 µL of the provided Speedy Supreme NZYTaq 2x MM Reconstitution Buffer. Mix gently by pipetting up and down, followed by a light flick. Allow the mixture to stand for about 1 minute, then flick again. Spin down briefly to collect the solution at the bottom of the tube. Do not replace the provided Speedy Supreme NZYTaq 2x MM Reconstitution Buffer with water or any other buffer.

  Note: *Speedy Supreme NZYTaq 2x MM Reconstitution Buffer contains DMSO. Upon freezing the reconstituted mix may form bubble-like structures. This does not compromise the efficacy of the product, however if it does occur allow the master mix to thaw completely, mix thoroughly, spin down, and then it is ready to use.*

Procedure

1. In a DNA and DNase-free microcentrifuge tube, combine the following components from the kit for lysing a single sample:
   - 20 µL Speedy Lysis Buffer
   - 0.5 µL Speedy Lysis Enzyme Mix

   Notes:
   - *Please notice that the Speedy Lysis Enzyme Mix may display slight precipitation; however, this will not compromise the efficacy of the kit.*
   - *For multiple samples, consider preparing a premix (e.g., 200 µL Speedy Lysis Buffer + 5 µL Speedy Lysis Enzyme Mix) that is stable for at least 1 h at room temperature (+18°C to +25 °C).*
   - *For blood samples, use 50 µL Speedy Lysis Buffer and 0.5 µL Speedy Lysis Enzyme Mix for one standard reaction.*
2. Insert an appropriate mouse typing sample into the lysis tube:
   - Mouse Tail: ~1 mm from the tail end.
   - Ear Punch: ~1 mm diameter. Avoid punches smaller than 0.3 mm for precision and larger than 1.5 mm to prevent reaction overload.
   - Mouse Hair: Include one tuft (approximately 3–30 hairs), ensuring placement at the tube bottom, roots down.
   - Mouse Blood: Add 1 to 2 µL of blood (fresh or frozen; collected in EDTA, citrate, heparin, or untreated) into the 50.5 µL of the lysis reaction.
3. Perform Sample Lysis during a 2-minute incubation with shaking at ambient temperature (+18°C to +25 °C). Shake vigorously by flicking the tube. An initial shaking is mandatory even if continuous shaking is not possible. Increasing incubation times up to 5 min may increase yield.

   Note: *For difficult samples vortex vigorously and extend incubation times to 5 minutes.*
4. Proceed with Enzyme Mix Inactivation for 3 minutes at +98 °C.
5. **Optionally**, centrifuge the lysate briefly to sediment debris. Centrifugation is typically not essential. Residual tissue may be visible but will not interfere with subsequent reactions.
6. The lysate can be stored as follows:
   - 1 week at room temperature (+18°C to +25 °C).
   - 1 month at +2°C to +8 °C.
   - 1 year at -85 °C to -15 °C. Repeated freeze-thaw cycles may decrease performance.
7. Typically use 5 µL of the lysate as the template for the subsequent PCR reaction, performed in a 25 µL final volume (you may use template volumes ranging from 1 to 7.5 µL in a 25 µL reaction).
8. A 25 µL reaction represents the recommended standard volume for the Lyo Speedy NZY Direct Genotyping Kit. Due to the kit's hot-start technology, reaction setup can occur at room temperature (+18°C to +25 °C). Gently mix and briefly centrifuge the master mix after thawing. A single reaction mixture of 25 µL should combine the following components. Please add components in the Table order. ***Note****: template lysate should be the last component to be added to the reaction mixture.*

COMPONENT	FINAL VOLUME/CONCENTRATION
Nuclease-free water	up to 25 µL
Reconstituted Lyo Speedy Supreme NZYTaq 2x Master Mix	12.5 µL
Primers	Primers 0.25 (0.15-0.45) µM (*)
Template	5 µL lysate (1 to 7.5 µL in 25 µL rxns)

(*) For duplex PCR, adjust primer concentrations (see note below).

9. Perform PCR using the following cycling parameters (ideally templates should be less than 1000 bp in size):

CYCLES	TEMP.	TIME	CYCLE STEP
1	95 °C	3 min	Initial denaturation
30-35(**)	94 °C	2 sec	Denaturation
	(*)	5 sec	Annealing
	72 °C	5 sec	Extension
1	72 °C	1 min	Final Extension

(*) Annealing temperature should be optimized for each primer set based on the primer T_m; typically it should be T_m-5 °C.
(**) Total PCR time for 35 cycles is 44 min.

10. Separate the PCR products through agarose gel electrophoresis (1 - 1.25 %, w/v) and visualize with GreenSafe Premium (MB13201) or any other means. There is no need to add loading dye for gel electrophoresis because the PCR mix already contains a blue dye and suitable density.

Note: *The blue dye present in the reconstitution buffer migrates similarly to DNA of 3000 bp. Thus, monitor the sample migration accordingly.*

Direct PCR procedure

For the direct PCR protocol, place the sample, ±1 mm diameter ear punch, ±1 mm outer tail clipping, a small tuft of hair, approximately 3–30 hairs, or 1 µL of blood sample, directly into the PCR reaction (50 µL of volume). For direct PCR a 50 µL volume PCR reaction is highly recommended. Adjust reaction components accordingly.

Technical Notes

Primer Design

Crafting primers that are optimal for your PCR necessitates a blend of accurate, meticulous design and adherence to foundational biochemical principles. PCR primers generally range in length from 15–30 bases and are designed to flank the region of interest. Primers should contain 40–60 %GC to ensure primer stability and specificity, and care should be taken to avoid sequences that might produce internal secondary structures. Ensure the 3´-ends of your primers are not complementary to each other, avoiding primer-dimer formation. Primer dimers unnecessarily remove primers from the reaction and result in an unwanted polymerase reaction that competes with the desired reaction. Avoid three G or C nucleotides in a row near the 3´-end of the primer, as this may result in non-specific primer annealing, increasing the synthesis of undesirable reaction products. Ideally, both primers should have nearly identical melting temperatures (T_m); in this manner, the two primers should anneal at roughly the same temperature.

Amplicon size

Animal/mouse genotyping commonly targets amplicons <1 kb, striking a balance between sufficient informational content and amplification efficiency. For amplicons exceeding 2 kb, employing crude extraction methods is not advisable, and one should opt for purified genomic DNA (may use NZY Tissue gDNA Isolation kit, MB135).

Multiplex assays

Multiplex assays with up to three primer pairs can be performed with the Lyo Speedy NZY Direct Genotyping Kit, provided that the annealing temperature and relative primer concentrations have been optimized. Multiplex PCR is likely to require higher $MgCl_2$ concentrations than singleplex PCR. We recommend using purified mouse genomic DNA as a template for multiplex assays with more than three primer pairs.

Quality control assays

Genomic DNA Contamination

Lyo Speedy Supreme NZYTaq 2x Master Mix and Speedy Supreme NZYTaq 2x MM Reconstitution Buffer must comply to internal standards of DNA contamination as evaluated through polymerase chain reaction (PCR).

Nucleases assay

Speedy Lysis Enzyme Mix, reconstituted Lyo Speedy Supreme NZYTaq 2x Master Mix and Speedy Supreme NZYTaq 2x MM Reconstitution Buffer are tested for nuclease contamination. To test for DNase contamination, 0.2-0.3 µg of pNZY28 plasmid DNA are incubated with the samples for 14-16 h at 37 °C. To test for RNase contamination, 1 µg of RNA is incubated with the enzyme 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

Lyo Speedy NZY Direct Genotyping Kit is tested for performance in a polymerase chain reaction (PCR) using blood samples.

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.

NO AMPLICON DETECTED
• **Unfavourable primer selection.**
Confirm the specificity and efficacy of the primers by ensuring their ability to amplify the target from 1–10 ng of purified genomic DNA. Evaluate primer annealing temperatures through a gradient PCR.
• **Inappropriate storage conditions**
After resuspension, store Resuspended Lyo Speedy Supreme NZYTaq 2× Master Mix away from light, preferably within the product box in a freezer (-35 °C to -15°C). Limit exposure to ambient lab lighting and avoid direct sunlight.
• **Excessive extract in PCR**
You may use up to 30% of lysate in a PCR reaction. In the case of lysate inhibition, this value should optimally be reduced to 4 to 10% per reaction, corresponding to 1 to 2,5 µL of lysate per 25 µL reaction.
• **Omission of Enzyme Mix or heat incubation step.**
Ensure the addition of Speedy Lysis Enzyme Mix to the Buffer and verify the execution of a minimum 2 min incubation at +98 °C to inactivate the enzyme mixture.
• **Suboptimal PCR cycling conditions.**
Explore varying the annealing temperature, adjusting extension time, or increasing the cycle number up to 40 to enhance amplification.
• **Contamination with DNases.**
Ensure that all labware, including pipettes, tubes, and containers, is clean and free from residual DNase contamination. Use DNase-free, autoclaved, or sterile equipment whenever possible. Use DNase-free water. Change gloves frequently. If you need to store extract samples for an extended period, consider freezing them at -20 °C or even at -80 °C; as this can help prevent DNase activity.
LOW AMPLICON YIELD.
• **Ineffective annealing temperature and extension time.**
Optimize the PCR protocol by tweaking the annealing temperature and extension time, while observing the impact on amplicon yield.
AMPLICON INCORRECT SIZE OR UNEXPECTED AMPLICON NUMBERS.
• **Primer misdesign or misbinding.**
Verify the design of the primers and validate their ability to selectively amplify the desired fragment from 1-10 ng of purified genomic DNA. Ensure specificity through *in silico* PCR and validate with control DNA.
• **Cross-contamination.**
Create separate work areas for sample lysis and PCR setup. Use separate, dedicated pipettes, and disposable tips for each setup. Always follow good laboratory practices to avoid contamination. Include negative controls (no DNA template) in PCR reactions to monitor for contamination in reagents or labware.
EXPECTED TWO AMPLICONS, BUT ONLY ONE BAND IS OBSERVED.
• **Insufficient resolving power in the analysis method.**
Ensure that the chosen analysis method can discern between the two expected DNA fragment sizes. Consider utilizing a superior resolution or enhancing gel electrophoresis resolution by increasing run time or altering gel concentration.
• **Differences in amplification efficiency between primer pairs.**
Validate that both primer pairs exhibit comparable amplification efficiencies. If disparities are observed, cautiously titrate down the primer pair yielding an amplicon by employing a reduced concentration, thereby balancing the amplification outputs of the two target sizes.