MB475_IFU_EN_V2301

Speedy NZY Direct Genotyping Kit

Catalogue number	Presentations
MB47501	100 rxns
MB47502	500 rxns

Description

Engineered to process an array of tissue materials, including blood and non-invasive hair samples, this kit promises a seamless, animal-friendly genotyping experience. The Speedy NZY Direct Genotyping Kit integrates a streamlined one-tube sample preparation with a swift PCR process, enabling rapid lysis of biological samples, including mouse genotyping tissues samples (tail clipping and ear punch), in under 5 minutes. The kit is validated for hair sample genotyping, which executed with minimal animal discomfort and without necessitating anaesthesia harmoniously combines ethical and efficient genotyping. The Speedy NZY Direct Genotyping Kit includes a potent Speed 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 Speedy Supreme NZYTaq 2x Green 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 contains a green dye for direct loading onto agarose gels, omitting the need for additional dye application. The 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 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

Speedy NZY Direct Genotyping Kit can be shipped on dry ice to blue ice. 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 Speedy NZY Direct Genotyping Kit delivers consistent and reliable results across its lifespan and usage. All components are formulated to be ready to use.

Note: Please notice that the Speedy Lysis Enzyme Mix may display slight precipitation; however, this will not compromise the efficacy of the kit.

Components

COMPONENT	MB47501 (100 rxns)	MB47502 (500 rxns)
Speedy Lysis Buffer	5 mL	5 × 5 mL
Speedy Lysis Enzyme Mix	50 µL	5 × 50 µL
Speedy Supreme NZYTaq 2x Green Master Mix	1250 µL	5 × 1250 µL

Sample Lysis and Product Applications

Expertly formulated for the rapid extraction and amplification of DNA from mouse tissues, the 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 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 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.

1. In a DNA and DNase-free microcentrifuge tube add the following:

- 20 µL Speedy Lysis Buffer
- 0.5 µL Speedy Lysis Enzyme Mix

Note 1: For multiple samples, consider preparing a premix (e.g., 200 µL Buffer + 5 µL Enzyme Mix) that is stable for at least 1 h at room temperature (+18°C to +25 °C).

Note 2: 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 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 (**Note**: template lysate should be the last component to be added to the reaction mixture; add components in the Table order):

COMPONENT	FINAL VOLUME/CONCENTRATION
Nuclease-free water	up to 25 µL
Speedy Supreme NZYTaq 2× Green 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):

CYCLE STEP	TEMPERATURE	TIME	Nº CYCLES
Initial denaturation	95 °C	3 min	1
Denaturation	94 °C	2 s	30-35**
Annealing	(*)	5 s	
Extension	72 °C	5 s	
Final Extension	72 °C	1 min	1

** 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 dye and suitable density.

Direct PCR

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.

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). Speedy Supreme NZYTaq 2× Green Master Mix, for instance, can amplify fragments up to 6 kb from purified mouse genomic DNA (when using 10s/kb of extension time). For GC-rich amplicons (>65% GC content), you might consider supplementing reactions with 5% DMSO to aid amplification.

Multiplex assays

Multiplex assays with up to three primer pairs can be performed with the 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 template for multiplex assays with more than three primer pairs.

Quality control assays

Nucleases assay

Speedy Lysis Enzyme Mix is tested for nucleases contamination. To test for DNase contamination, 0.2-0.3 µg of pNZY28 plasmid DNA are incubated with the speedy lysis enzyme mix 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.

Genomic DNA Contamination

Speedy Supreme NZYTaq 2x Green Master Mix must comply to internal standards of DNA contamination as evaluated through real-time qPCR.

Functional assay

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.

1. **No amplicon detected.**

   a. 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.

   b. Inappropriate storage conditions.

   Store Speedy Supreme NZYTaq 2× Green 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.

   c. 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.

   d. 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.

   e. Suboptimal PCR cycling conditions.

   Explore varying the annealing temperature, adjusting extension time, or increasing the cycle number up to 40 to enhance amplification.

   f. 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; this can help prevent DNase activity.

2. Low amplicon yield.

a. 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.

3. Amplicon incorrect size or unexpected amplicon numbers.

a. 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.

b. 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.

b. Expected two amplicons, but only one band is observed.

b1. 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.

b2. 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.