



GENE CHECK, INC.

M2B2 PROTOCOL

Assay Set Up

Introduction

Gene Check's M2B2 will bind mismatch-containing DNA from any source, including synthetic oligonucleotides and amplified genomic DNA. Fragment lengths of 100 to 300 base pairs appear to work best, although shorter (down to 30 base pairs) and longer (up to 600 base pairs) fragments have been tested successfully.

Buffer and Sample Preparation

1. M2B2 wash buffer is PBS (pH 7.4). Each sample will require approximately 3 ml of wash buffer.
2. DNA/ streptavidin-HRP dilution buffer is PBS + 1% BSA. Up to 200 μ l of dilution buffer is required for each test sample. After BSA is dissolved in PBS, the solution should be filtered through a 0.45 μ m filter.
3. Test DNA must be end-labeled. At Gene Check, we prefer to use biotin, which is detected with a streptavidin-conjugated horseradish peroxidase (HRP) and tetramethyl-benzidine (TMB).
4. The amount of test DNA which is optimal for the M2B2 assay must be determined empirically and is dependent on the length and nature of the fragment being examined. Generally, DNA amounts in the 1-100 ng range give good positive signals and good discrimination between mismatched and nonmismatched DNA. It is advisable to test a number of DNA concentrations when first working with a particular DNA fragment.
5. DNA should be diluted in DNA dilution buffer to 15-190 μ l. In general, smaller volumes give stronger signals.

DNA Binding

1. All steps are performed at room temperature. Do not let M2B2 dry at any step.
2. Vortex M2B2 to resuspend.
3. Add 10 μ l of M2B2 to each test sample.
4. Note: Some applications may require more M2B2.
5. Agitate tubes individually by "flicking" with fingers. Creation of air bubbles during agitation appears to increase mixing.
6. Incubate tubes for at least 30 minutes with agitation (shaking, rocking, or rotating). Longer incubation times (up to 2 hours) may increase signal.
7. Following incubation, centrifuge tubes briefly and remove supernatant.

Washing

1. Add 500 μ l wash buffer to each tube. Move the M2B2 through the buffer several times by rotating the tubes in front of the magnet.
2. Apply magnet.
3. Remove supernatant.
4. Repeat wash steps 1-3.

Detection of DNA

The method of DNA detection employed will depend on the label employed and on the preferences of the individual researcher. At Gene Check, we use biotin- labeling and the following detection protocol:

1. Add 100 μ l 1.0 μ g/ml streptavidin-HRP in PBS + 1% BSA (DNA dilution buffer) to each sample.
2. Resuspend M2B2 by flicking.
3. Incubate for 20 minutes with agitation (shaking, rocking or rotating).
4. Centrifuge briefly.
5. Apply magnet.
6. Remove supernatant.
7. Add 500 μ l wash buffer each sample.
8. Invert tubes and shake forcefully.
9. Centrifuge briefly.
10. Apply magnet and remove supernatant.
11. Add 500 μ l wash buffer and transfer wash with M2B2 to a fresh tube (this step greatly reduces residual background signals caused by DNA and/or conjugate adhering to the plastic). An additional wash is optional.
12. Apply magnet and remove all the wash buffer.
13. Add 100 μ l TMB and incubate for 5-30 minutes. Stop the reaction with 100 μ l 0.5M H₂SO₄.
14. Read at 450 nm.