

FlashPlate® File #5

Evaluation of FlashPlate in a Helicase Assay

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Abstract

This study demonstrated the efficacy of the FlashPlate as a high throughput screening (HTS) platform for a helicase functional assay. Helicase activity was measured on a streptavidin coated FlashPlate (SMP103), using two DNA oligos. One of the oligos was biotinylated; the other was 5' end-labeled with [³³P]-γ-ATP. The oligos were preannealed to each other, to form a [³³P]-biotinylated DNA duplex substrate, then immobilized on the SMP103 FlashPlate. The FlashPlate platform provided an effective and economical basis for the evaluation of DNA helicase enzyme activities.

Introduction

Helicases are enzymes that catalyze the unwinding of DNA strands, promoting replication and playing an important role in DNA repair. Conventional helicase functional assays require that unwound DNA products be analyzed on polyacrylamide gels, which have major constraints in high throughput screening (HTS) applications. Preparing the gels is a time-, labor-, and material-intensive task, especially at high volume.

FlashPlate is a 96-well polystyrene microplate with plastic scintillator coated wells, available exclusively from PerkinElmer Life Sciences. The platform is compatible with automated assay equipment such as the TopCount® Microplate Scintillation and Luminescence Counter from Packard Instrument Company.

Materials

- Streptavidin FlashPlate PLUS (SMP103)
- Oligonucleotides

#1 (60 mer)

5' biotin-GGTTTAAAAA ATAGGAGGGA CAACGTCGTG
ACTGGGAAAA CTCCCCGGGT ACCGAGCTCG-3'

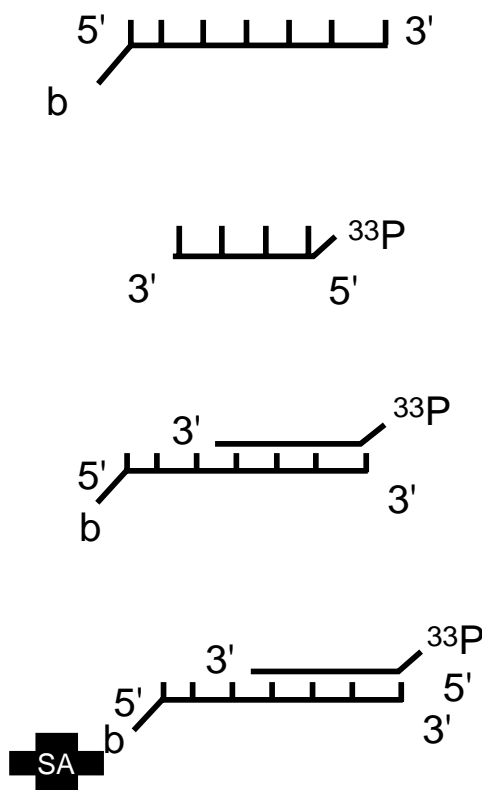
#2 (22 mer)

5'-GTTTTCCCAG TCACGACGTT GT-3'

- SV40 Large T-Antigen (Molecular Biology Resources Inc., Milwaukee, WI)
- Assay Buffer (50 mM Tris HCl, pH 7.5, 50 mM NaCl, 1 mM DTT, 0.5 mM MnCl₂, 20% glycerol and ± 1 mM ATP)
- Polynucleotide Kinase (T4) (NEE101)
- [³³P]-γ-ATP (NEG602H)

Procedure

Figure 1



1. DNA oligo #2 was 5'-labeled with ^{33}P by T4 polynucleotide kinase, following the manufacturer's protocol.
2. Equal molar quantities of DNA oligo #1 and the [^{33}P]-labeled #2 oligo were annealed at 90°C for 5 minutes and cooled to room temperature.
3. Approximately 1 ng of DNA duplex was applied to each well of the SMP103 FlashPlate in phosphate buffer containing 1 M NaCl (pH 7.4) and incubated for a minimum of 4 hours at room temperature or, alternately, overnight at 4°C. Each well was washed twice with 200 μl of PBS at room temperature, and once with 200 μl of 50 mM Tris (pH 7.5), 50 mM NaCl at 37°C. Each well contained approximately 10,000 to 12,000 CPM.
4. The coated plate was assayed with various amounts of SV40 Large T-Antigen (0 μg , 0.7 μg , 1.4 μg , 2.8 μg) in 50 μl of assay buffer, in the presence or absence of 1 mM ATP. The reaction was incubated at 37°C and monitored in real time on a microplate scintillation counter, as indicated in Figures 2 and 3. Percentage of unwinding was calculated as follows:

$$\% \text{ unwinding} = \frac{[(\text{CPM} @ \text{Time}_{(0)}) - \text{CPM} @ \text{Time}_{(x)}] / \text{CPM} @ \text{Time}_{(0)}}{1} \times 100$$

See Figure 1.

Results and Discussion

In the presence of ATP, double stranded DNA duplex can be unwound by T-Antigen (0.7 μg) beginning at the single/double strand junction, resulting in the release of the [^{33}P]-labeled DNA strand (22 mer). As negative controls, the assay was also performed in the absence of ATP, and in the absence of T-Antigen, the results of which demonstrated that decreases in CPM were specific to T-Antigen helicase activity, and not a consequence of temperature-induced melting or nuclease activity (Figure 2). Furthermore, the level of DNA unwinding activity was shown to be dependent upon the dosage of T-Antigen added (Figure 3).

FlashPlate allowed the investigator to quantify enzyme activities, and to follow enzyme kinetics in real-time. Similar assays have been performed using other helicases (e.g., HSV helicase - primase) using appropriate assay conditions and nucleic acid substrates. The efficacy of FlashPlate for HTS assays of helicases was demonstrated.

Figure 2

DNA Unwinding is ATP Dependent

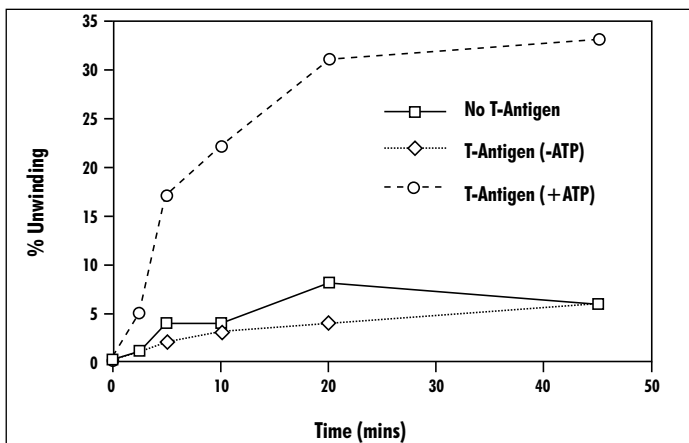
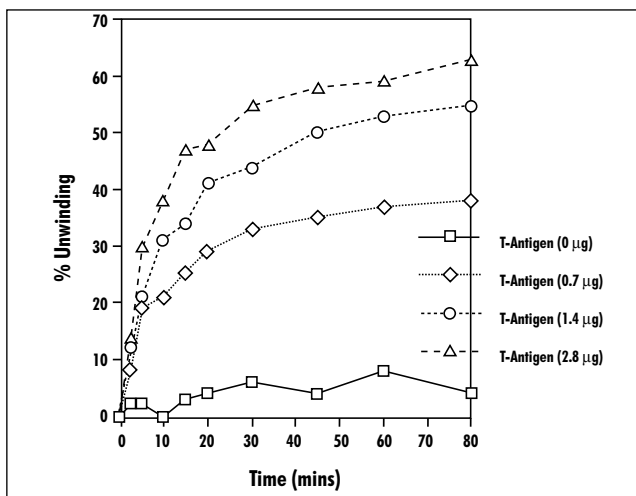


Figure 3

DNA Unwinding is Proportional to Added T-Antigen



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