EURx Molecular Biology Products

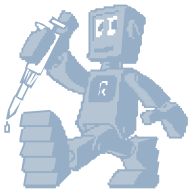
SV40 DNA REPLICATION ASSAY KIT

INSTRUCTION MANUAL
VERSION 1.7.01

CATALOG NUMBER E8050-01
20 REACTIONS

STORAGE CONDITIONS
STORE AT -80^{O}C

Distributor:



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Not for drug, household or other uses.

EURx Molecular Biology Products

TECHNICAL DATA SHEET

SV40 DNA Replication Assay Kit

Catalog Number: E8050-01 20 Reactions

Lot Number: 2003020

HeLa Extract Lot Number: 2912015
Amount of HeLa Extract needed per replication assay is 10.0 µl
Store at –80º C

Plasmid DNA pUC HSO (Ori +) Lot Number: 190302
Amount of pUC HSO needed per replication assay is 1.0 µl

Plasmid DNA pUC 8-4 (Ori -) Lot Number: 190303
Amount of pUC 8-4 needed per replication assay is 1.0 µl

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I. INTRODUCTION

Viral model systems have greatly aided the study of DNA replication in both prokaryotes and eukaryotes (1). For the latter, the workhorse has been the SV40 system, which was first described by Joachim Li and Thomas Kelly in 1984 (2,3). This system was a significant advance because it used DNA with a well-defined origin of replication and required only one virus-encoded protein, the SV40 large T antigen, all other essential proteins being supplied by an extract prepared from the monkey or human host cell. The SV40 system has been subsequently dissected and developed to the point where it can now be completely reconstituted with purified human proteins (4-7). The usefulness of the SV40 DNA replication system has also been extended to applications such as cell cycle control (8,9) and the fidelity of DNA replication (10).

The EURx SV40 DNA Replication Assay Kit is designed to evaluate the ability of T antigen to promote the replication of SV40 origin-containing DNA. This is done by comparing the amount of DNA synthesized in reactions containing a plasmid with an intact SV40 origin of replication (pUC HSO) with that synthesized in reactions with an origin-defective plasmid (pUC 8-4). The difference represents T antigen-dependent DNA replication. The kit can be used to evaluate the researcher's own T antigen, or alternatively, be used as a set of positive controls for the other components of the replication assay.

The SV40 DNA Replication Assay Kit includes plasmid DNA, nucleotides, buffers, and HeLa cytoplasmic extract. SV40 large T antigen is available separately from EURx (Catalog Number E5800).

II. COMPONENTS

A. STORAGE AND STABILITY

The kit should be stored at -80°C. The reagents are stable for at least 6 months. **Once thawed, aliquots of the HeLa extract that are not used should be discarded.** Use of refrozen extract will result in diminished performance of this assay system.

B. REAGENTS PROVIDED WITH THE KIT

Plasmid DNA pUC HSO (Ori+)	See Tech. Sheet
Plasmid DNA pUC 8-4 (Ori-)	See Tech. Sheet
HeLa extract	See Tech. Sheet
10X Reaction buffer	60 µl
20X dNTPs/NTPs	30 µl
1 M Phosphocreatine	30 µl
Creatine phosphokinase, 625 units/ml	25 µl
Autoclaved water	100 µl
Yeast RNA co-precipitant	1250 µl

C. MATERIALS NOT PROVIDED WITH THE KIT

[α-^{32}P or ^{33}P]dCTP, 10 mCi/ml, approximately 1000 Ci/mmol
SV40 Large T-Antigen, *EURx* Catalog Number E5800
10% Trichloroacetic acid (TCA)
95% Ethanol
Vacuum filter apparatus
Glass fiber filters (Whatman GF/C or equivalent)
Scintillation fluid
Liquid scintillation counter

III. PROTOCOL

A. ASSEMBLY OF SV40 DNA REPLICATION REACTION:

Prepare the reagents not supplied with the kit (see Materials Not Provided With The Kit). Dilute the [α-^{32}P or ^{33}P]dCTP 10X in water, e.g., 1 µl of isotope to 9 µl of water.

Thaw the kit reagents and place them on ice. Use a separate 1.5 ml microcentrifuge tube for each reaction. See the Technical Analysis sheet included with the kit for the lot-specific amount of HeLa extract and plasmid DNA to add to each replication reaction. Assemble the following components in the indicated order for both the ori+ and the ori- reactions:

Reagents	**ori+**	**ori-**
10X Reaction buffer	2.5 µl	2.5 µl
20X dNTPs/NTPs	1.25 µl	1.25 µl
Plasmid DNA	See Technical Analysis Sheet for specific volume	
1 M Phosphocreatine	1.0 µl	1.0 µl
Creatine phosphokinase, 625 units/ml	1.0 µl	1.0 µl
SV40 large T antigen	approx. 1 µg	approx. 1 µg
HeLa extract	See Technical Analysis Sheet for specific volume	
10X diluted [α-^{32}P or ^{33}P]dCTP	1.0 µl	1.0 µl
Water	Bring to final volume	Bring to final volume
Final reaction volume	25 µl	25 µl

B. FINAL REACTION CONCENTRATION: 30 mM HEPES, pH 7.5, 7 mM $MgCl_2$, 0.5 mM dithiothreitol, 4 mM ATP, 100 µM each of dATP, dGTP, dTTP, dCTP, 50 µM each of CTP, GTP, UTP, 40 mM phosphocreatine, 0.625 units creatine phosphokinase, 1 µCi dCTP, 50 ng plasmid DNA, 1 µg T antigen (recommended amount), and HeLa extract as per Technical Analysis sheet.

C. DETERMINATION OF SPECIFIC RADIOACTIVITY (CPM/PMOL dCTP): Remove 1 μl of each complete reaction mixture and place it onto a separate filter disk. Transfer the disk to a scintillation vial and fill the vial with scintillation fluid. Count the vial in a liquid scintillation counter.

D. DNA REPLICATION REACTIONS: The remaining reaction mix is incubated at 37°C for 4 hours. At the end of the incubation period immediately add 50 μl of yeast RNA co-precipitant and 1.0 ml of 10% TCA. Chill the reaction tubes on ice for at least 10 min. Filter the contents of the reaction tube through a filter disk on a vacuum filter apparatus. Wash the disk thoroughly with 10% TCA followed by 95% ethanol. Transfer the dried filter disk to a scintillation vial and fill the vial with scintillation fluid. Count the samples in a liquid scintillation counter.

IV. CALCULATIONS

Specific radioactivity (i.e., cpm/pmol dCTP for ori+ and ori- reaction mixtures)

SRA = cpm/pmol dCTP
= (cpm for 1μl ori+ reaction)/100 pmoles dCTP in 1μl of reaction mix

pmoles synthesized for ori+ reaction

pmoles synthesized for ori+ reaction =
(cpm for ori+ reaction filter) (1/SRA) (4 pmoles nucleotide/ pmole dCTP)

pmoles synthesized for ori- reaction

pmoles synthesized for ori- reaction =
(cpm for ori- reaction filter) (1/SRA) (4 pmoles nucleotide/ pmole dCTP)

Net pmoles of SV40 origin-dependent DNA synthesis

net pmoles synthesized = (pmoles ori+) - (pmoles ori-)

Example:

1 μl ori+ reaction mix cpm = 108,400 cpm
1 μl ori- reaction mix cpm = 107,000 cpm

ori+ precipitated filter cpm = 23,577 cpm
ori- precipitated filter cpm = 3,210 cpm

Calculation steps:

A. SRA for ori+ reaction =
108,400 cpm/100 pmoles dCTP = 1084 cpm/pmol dCTP

B. SRA for ori- reaction =
107,000 cpm/100 pmoles dCTP = 1070 cpm/pmol dCTP

C. pmoles synthesis for ori+ reaction =
(23,577cpm) (1pmol dCTP/1084 cpm) (4 pmoles nt/pmol dCTP) = 87

D. pmoles synthesis for ori- reaction =
(3210 cpm) (1 pmol dCTP/1070 cpm) (4 pmoles nt/pmol dCTP) = 12

E. Net pmoles of SV40 origin-dependent DNA synthesis = 87 – 12 = 75

V. REFERENCES

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VI. RELATED PRODUCTS

SV40 Large T Antigen	*EURx* Catalog No. E5800
DNA Polymerase Alpha, Human	*EURx* Catalog No. E1075