For Research Use Only Not for Diagnostic Use

HIV-1 Integrase Assay Kit

Version 3.0
Catalog Number EZ-1700

INTRODUCTION

HIV DNA is integrated into host DNA by a viral encoded integrase and this enzyme activity is a likely target site in the HIV life cycle to block viral infection.

The XpressBio HIV-1 Integrase Assay Kit is a non-radioactive assay used to quantitatively measure integrase activity, the effects of interacting proteins, anti-viral compounds, and other test articles on HIV-1 integrase activity. Streptavidin-coated 96-well plates are coated with a double-stranded HIV-1 LTR U5 **d**onor **s**ubstrate (DS) DNA containing an end-labeled biotin. Full-length recombinant HIV-1 integrase protein is loaded onto the DS DNA substrate. Integrase test articles are added to the enzyme reaction and then a different double-stranded **t**arget **s**ubstrate (TS) DNA containing a 3'-end modification is added to the reaction mixture. The HIV-1 integrase cleaves the terminal two bases from the exposed 3'-end of the HIV-1 LTR DS DNA and then catalyzes a strand-transfer recombination reaction to integrate the DS DNA into the TS DNA. The products of the reaction are detected colorimetrically using an HRP-labeled antibody directed against the TS 3'-end modification. Sodium azide is included in the kit as a positive control compound that inhibits HIV-1 integrase catalytic activity.

KIT CONTENTS

Product	Catalogue	Per Kit
Reaction Buffer	EZ-1701	240 mL
Blocking Buffer	EZ-1702	30 mL
DS Oligo DNA 100X Solution	EZ-1703	120 µL
HIV-1 Integrase (2 µM)	EZ-1704	70 µL
Sodium Azide (20%)	EZ-1705	1.8 mL
TS Oligo DNA 100X Solution	EZ-1706	65 µL
HRP Antibody Solution	EZ-1707	12 mL
Dilution Plate	EZ-1708	1 plate
Streptavidin-coated 96-well Plate	EZ-1709	1 plate
Wash Buffer Concentrate 20X	82710	60 mL
TMB Peroxidase Substrate	XB-1006	12 mL
Stop Solution	XB-1007	12 mL
Instruction Manual	NA	1

TECHNICAL ASSISTANCE

Please refer any technical questions to info@xpressbio.com.

SAFETY INFORMATION

Sodium azide may react with lead and copper plumbing to form explosive azide compounds. When disposing of reagents, flush with copious quantities of water. The MSDS for this kit is available online at www.xpressbio.com.

STORAGE CONDITIONS

The streptavidin-coated plate, reaction buffer, block buffer, sodium azide, HRP antibody, TMB, wash solution, and the stop solution should be placed at 2-8ºC. The DS and TS DNAs, and the integrase enzyme should be stored at -20ºC or cooler. The kit is stable for one year under these conditions.

Microwell strips of the streptavidin-coated plate that are not used after opening the foil pouch should be returned to the pouch along with the sachet of desiccant, closed with a pouch sealer or adhesive tape, and stored at 2-8ºC until the expiration date on the label.

Reaction buffer (2 ml per reaction well) should be activated by the addition of β–mercaptoethanol (BME, 0.4 µl/1 ml) just prior to assay. BME-activated reaction buffer is stable for one week at 2-8ºC. Reaction buffer may settle during storage, so the bottle should be warmed to 37º C and mixed before use.

REAGENTS AND EQUIPMENT SUPPLIED BY THE USER

- Pipettors and sterile tips
- Disposable gloves
- 14.5 M β-mercaptoethanol (BME)
- Paper towels
- Sterile distilled (deionized) water
- A 37ºC incubator
- A 37ºC water bath
- A 96-well plate reader capable of reading at 450 nm

NOTES BEFORE STARTING

General Comments

Carefully review the protocol before beginning, since small deviations may lead to discrepancies in the final results. All incubation steps should be performed within +/- 2 min of the indicated times.

Each lot of ExpressBio HIV Integrase Assay Kit has been extensively tested and the conditions under which the kit is shipped and stored have been shown empirically to not impact assay performance.

As with all other 96-well applications, there may be a slight difference in assay performance when the plate outer wells, and especially the plate corners are used. Consequently, the inner wells of the plate should be used preferentially in your experimental design whenever critical results are required.

Wash Buffer Concentrate

Wash buffer is provided as a 20X concentrate. Mix 50 mL of 20X wash buffer concentrate with 950 mL sterile distilled water to make a 1X wash buffer solution before use.

Reaction Buffer

Reaction buffer (1x) contains both manganese and magnesium, and requires the addition of 0.4 µl of BME/mL of reaction buffer before use. Prepare 2 ml of reaction buffer for each well. Reaction buffer is stable for one week stored at 2-8ºC after addition of BME. Add BME to the appropriate amount of buffer, do not activate the entire 220 ml bottle unless all will be used. The reaction buffer may settle in the bottle and it should be mixed/swirled gently before use.

DS and TS DNAs

The DS and TS DNAs are provided as 100X solutions that should be diluted 100-fold into reaction buffer before use. The diluted 1X DS and TS DNA solutions are not stable and the amount required for an experiment should be prepared just before use. The 100X stocks of these DNAs are virtually unaffected by multiple freeze-thaw cycles. The TS DNA is light-sensitive and may leech onto polypropylene tubes.

HIV-1 Integrase Protein Stability

The HIV-1 integrase protein should be stored at -20**º** C or colder and is stable to freeze-thaw cycles with 10-12 possible. Before each use the enzyme solution should be thawed on ice for five minutes and then re-frozen.

Plate Washing Steps

Washing can be done manually, with a wash bottle, or with an automatic plate washer. The liquid should be completely removed from the plate wells after each washing step by patting the plate down onto a stack of paper towels.

Replicates

We recommend all samples and controls are tested using 2-3 replicate wells in each experiment. The use of additional replicates for the blank (no integrase) and the integrase-alone controls (no test article) may reduce variability in the assay.

HIV INTEGRASE ASSAY KIT PROTOCOL

DS Coating and Blocking of SA Plate

1. Prewarm reagents

Place the reaction buffer and the blocking solution in a 37ºC water bath for 10 min before starting the assay. Prewarm all the other components of the kit except the HIV-1 integrase enzyme by placing them at room temperature.

2. Coat with DS DNA

Remove any strip wells from the streptavidin-coated 96-well plate that will not be used and reseal them in the foil pouch containing desiccant. Dilute the required amount of DS DNA 100X solution 100-fold in reaction buffer (10 µL DS DNA 100X solution and 990 µL reaction buffer). Add 100 µL of 1X DS DNA solution per well and incubate for 30 min in a 37ºC incubator. Return the reaction buffer to the 37ºC water bath.

3. Blocking the plate

Aspirate the liquid from the plate wells and wash five times with 300 µL 1x wash buffer. Add 200 µL of blocking buffer per well and incubate for 30 min in a 37ºC incubator.

If required, the plate may now be placed at 2-8ºC overnight and later placed in a 37ºC incubator for 20 min before continuing with the protocol, however the kit performs slightly better if the entire protocol is performed in one day.

Integrase Reaction

4. Load the integrase onto DS DNA

Thaw the HIV-1 integrase on wet ice or at 2-8ºC (~5 min) before it is needed and centrifuge the tube briefly (ex. 10,000 RPM X 5 sec) before use. Dilute the enzyme 1:300 into reaction buffer (2 µL HIV-1 integrase and 598 µL reaction buffer). Aspirate the liquid from the plate wells and wash three times with 200 µl reaction buffer. Add 100 µL of reaction buffer (negative control) or integrase enzyme solution (positive control) per well and incubate for 30 min in a 37ºC incubator. Include reaction buffer only replicates (without integrase) as a no enzyme negative control. Return the reaction buffer to the 37ºC water bath.

5. Add Inhibitors or Test Articles

Prepare test articles by diluting to 2X final desired test concentration in reaction buffer. For example, prepare a 20 µM or 20 µg/mL solution and serially dilute in reaction buffer when a 10 µM or 10 µg/mL high-test concentration is desired for the assay. Azide solution diluted to 0.30% (2X concentration or 0.15% final 1X concentration) inhibits approximately 50% of the integrase activity. Include 50 µL reaction buffer negative and positive control replicates (no test article) in each experiment. Test articles may contain up to 10% dimethyl sulfoxide (DMSO), since the integrase reaction is only marginally affected by the presence of up to 5% DMSO in the final reaction.

Aspirate the liquid from the plate wells and wash them three times with 200 µL reaction buffer. Add 50 µL per well of each test article in reaction buffer (reaction buffer alone for positive and negative controls) and incubate for 5 min at room temperature.

6. Add TS DNA

Dilute the required amount of TS DNA 100X solution 100-fold in reaction buffer (10 µL TS DNA 100X solution and 990 µL reaction buffer per mL). Add 50 µL of the 1X TS DNA solution per well directly to the 50 µL buffer/test articles already present in the wells. Mix the reactions by tapping the plate gently against a stationary hand 3-5 times. Incubate for 30 min at 37ºC.

Detection of Reaction Products

7. Add HRP Antibody

Aspirate the liquid from the plate wells and wash five times with 300 µL wash solution. Add 100 µL HRP antibody solution per well and incubate for 30 min at 37ºC.

8. Add TMB Peroxidase Substrate

Aspirate the liquid from the plate wells and wash five times with 300 µL wash solution. Add 100 µL TMB peroxidase substrate solution per well and incubate for **10 minutes** at room temperature.

9. Add TMB Stop Solution

Add 100 µL TMB stop solution directly to the wells containing the TMB substrate. Burst any large bubbles by using a pipette tip. Read the absorbance of the wells for a minimum of 0.1 sec using a plate reader set at 450 nm. Plates should be read within 10 min of adding TMB stop solution.

If the OD 450 nM absorbance is above the range of the plate reader, the reactions can be dilute in order to realize a more accurate reading or end point. Add 100 µl of the assay reaction wells to 100 µl of dH_2O into the dilution plate/strip or well (EZ-1709 provided, do not use the SA-coated plate/strips) and read the absorbance at 450 nM. The absorbance values from the diluted samples should be multiplied by 2.

DATA ANALYSIS AND ASSAY PERFORMANCE

Experimental data are analyzed as described below; typically, duplicate or triplicate determinations are obtained for each control/sample/drug/test-article in each experiment.

Determine the mean blank absorbance (reaction buffer negative control) in the assay from the no integrase/no test article control (usually less than 0.25 OD units) and subtract this background absorbance from the other readings.

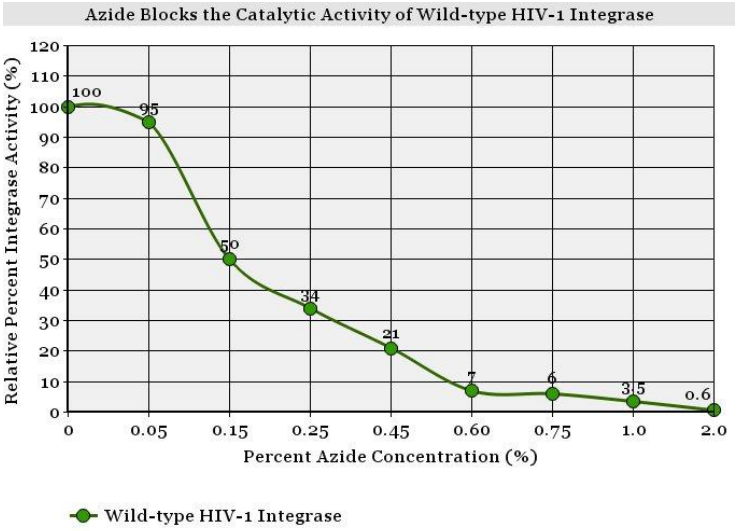
Calculate the mean +/- standard deviation (SD) and CV (SD/mean X 100%) for the background corrected absorbance of integrase alone (positive control) and test articles replicate wells.

Convert the data to percent control activity by dividing the mean absorbance of test articles by that of the integrase alone control and multiplying by 100%. The mean absorbance of the test articles divided by the mean integrase control activity multiplied by the associated CV provides the percent adjusted standard deviation. The CV = %SD for the 100% integrase alone control.

Typical assay results for buffer/no integrase, integrase alone/no azide, and integrase plus azide (each point run in triplicate) are tabulated below for the inhibition of the HIV-1 integrase when treated with azide:

Sample ID	Mean OD (Corrected)	Std. Deviation (Corrected)	% CV	% Control Activity
Buffer	0.000	0.007	---	---
Integrase	4.42	0.290	6.6%	100%
2.0% Azide	0.022	0.002	9.1%	0.5%
1.5% Azide	0.081	0.007	8.6%	1.8%
1.0% Azide	0.135	0.021	15.6%	3.5%
0.6% Azide	0.354	0.042	11.8%	8.0%
0.4% Azide	1.061	0.104	9.8%	24%
0.2% Azide	1.856	0.160	8.6%	42%
0.1% Azide	3.138	0.248	7.9%	71%
0.05%Azide	4.19	0.360	8.6%	95%

A graph illustrating the dosage-dependent inhibitory effect of azide on the catalytic activity of the HIV-1 integrase is shown below.



The inhibitory concentration of azide that reduces integrase activity by 50% (IC_{50}) may be interpolated from the curve manually or using more sophisticated programming. In this example, azide showed an IC_{50} of 0.15% and an IC_{90} of 0.57%.

A summary of the absorbance values observed for background-corrected integrase-alone (integrase) and for the IC_{50} and IC_{90} values for sodium azide observed in three independently-performed experiments are shown below.

Expt	Integrase Alone[a]	IC_{50} (% NaN3)	IC_{90} (%NaN3) .
1	2.9 +/- 0.08	0.152	0.568
2	2.8 +/- 0.16	0.143	0.537
3	2.6 +/- 0.05	0.124	0.613
All	2.77 +/- 0.12	0.140 +/- 0.012	0.573+/-0.031 .

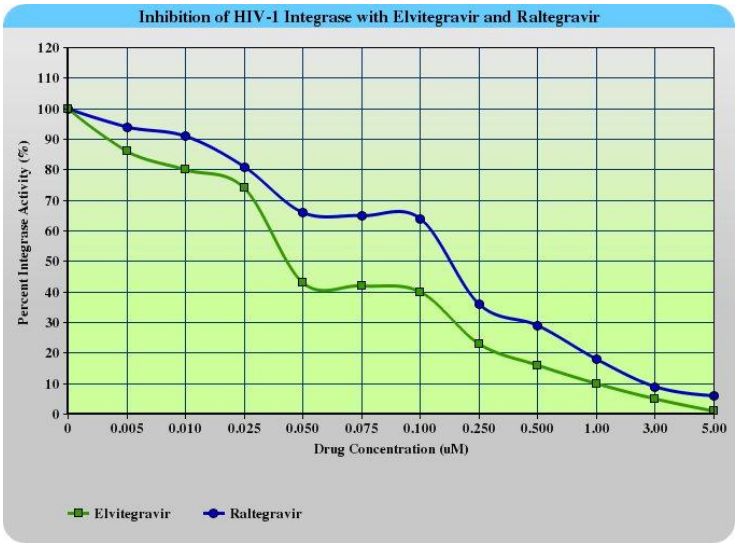
[a]Mean +/- SD background-corrected absorbance of six integrase alone control wells.

Note: The absorbance values generated by integrase alone may vary between experiments, but the IC_{50} and IC_{90} values are reproducible with CVs = 8.5% and 5.4%, respectively.

The data from a typical experiment with the HIV-1 integrase inhibitor Elvitegravir (EVG) are tabulated below:

Sample ID	Mean OD (Corrected)	Std. Deviation (Corrected)	% CV	% Control Activity
Reaction Buffer	0.000	0.007	---	---
Integrase Alone	4.37	0.133	3.0%	100%
3.0 µM EVG	0.225	0.019	8.4%	5.1%
1.0 µM EVG	0.426	0.009	2.1%	9.7%
0.5 µM EVG	0.692	0.093	13.5%	15.8%
0.25 µM EVG	1.020	0.021	2.1%	23.3%
0.10 µM EVG	1.80	0.225	12.5%	41.0%
0.05 µM EVG	1.90	0.277	14.6%	43.4%
0.01 µM EVG	3.49	0.316	9.1%	79.9%
0.005 µM EVG	3.78	0.397	10.5%	86.4%

A graph illustrating the dosage-dependent inhibitory effect of Elvitegravir and Raltegravir on the catalytic activity of HIV-1 integrase is shown below.



The inhibitory concentration of Elvitegravir and Raltegravir that reduces integrase activity by 50% (IC_{50}) and 90% (IC_{90}) may be interpolated from the data and the curve. In this experiment, Elvitegravir showed an IC_{50} of 40 nM and an IC_{90} of 975 nM, while Raltegravir showed an IC_{50} of 175 nM and an IC_{90} of 2.88 µM.

TROUBLESHOOTING GUIDE

Problem	Suggestion
Integrase alone signal >3.0	Dilute stopped reaction 1:1 in dH2O into a blank strip or well (do not use SA-coated plate). Dilute integrase enzyme at 1:350 in reaction buffer, see step 4 above.
Integrase alone signal <0.5	Spin down integrase before use. Dilute integrase enzyme 1:250 in step 4 above. Increase the TMB incubation time to 20-30 min.
Wells are stained blue	Add stop reagent before reading plates or read plate at 405 nM instead of 450 nM.
Background is high >0.35	Replace reaction buffer. Use reaction buffer within one week of adding BME. Swirl/mix the reaction buffer bottle before use. Review plate washing steps above.

EXPERIENCED USERS PROTOCOL

1. Prewarm reagents, 100 µL DS oligo, 30 min at 37ºC.
2. 5 X 300 µL wash buffer, 200 µL block, 30 min at 37ºC.
3. 3 X 200 µL reaction buffer wash, 100 µL of 1:300 dilution of integrase in reaction buffer, 30 min at 37ºC.
4. 3 X 200 µL reaction buffer wash, 50 µl reaction buffer or test article in reaction buffer, 5 min at room temperature.
5. 50 µL of TS oligo, 30 min at 37ºC.
6. 5 X 300 µL wash buffer, 100 µL HRP antibody, 30 min at 37º C.
7. 5 X 300 µL wash buffer, 100 µL/well of TMB substrate, 10 min at room temperature.
8. 100 µL TMB stop solution, read OD at 450 nM.

CONTRACT RESEARCH

Need a hand with your research? Would you like independent confirmation of your results? Why not let us perform the HIV-1 integrase assay for you? Contact us for more information.

CONTACT INFORMATION

Express Biotech International
4650 Wedgewood Blvd Ste 103
Frederick, MD, 21703 USA
Toll free: 888-562-8914

www.XpressBio.com
info@XpressBio.com
Tel: 301-228-2444
Fax: 301-560-6570