



HIV-1 Integrase Assay Kit

Version 2.0
Catalog Number EZ-1700

INTRODUCTION

The Xpress HIV-1 Integrase Assay Kit is used to quantitatively measure the effects of interacting proteins and other test articles on HIV-1 integrase activity. Streptavidin-coated 96-well plates are coated with a double-stranded HIV-1 LTR U5 donor substrate (DS) oligonucleotide containing an end-labeled biotin. Full-length recombinant HIV-1 integrase protein (200 nM, purified from bacteria) is then loaded onto this oligo substrate. Test articles are added to the reaction and then a different double-stranded target substrate (TS) oligo containing 3'-end modifications is added to the plate. The HIV-1 integrase cleaves the terminal two bases from the exposed 3'-end of the HIV-1 LTR DS and then catalyzes a strand-transfer reaction to integrate the DS into the TS. 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 activity.

SAFETY INFORMATION

Certain reagents in this kit contain sodium azide. Azides may react with lead and copper plumbing to form explosive azide compounds. When disposing of reagents, flush with copious quantities of water to minimize azide build up. The MSDS for this kit is available online at www.expressbiotech.com.

KIT CONTENTS

Product	Catalogue	Per Kit
Reaction Buffer	EZ-1701	220 mL
Blocking Buffer	EZ-1702	30 mL
DS oligo 100X solution	EZ-1703	120 µL
HIV-1 integrase (10 µM)	EZ-1704	200 µL
Sodium Azide (20 %)	EZ-1705	2 X 1 mL
TS oligo 100X solution	EZ-1706	60 µL
HRP antibody solution	EZ-1707	12 mL
Plate covers	EZ-1708	6 covers
Streptavidin-coated 96-well plates	EX8000-C96	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.

STORAGE CONDITIONS

If the kit will not be used within one week following arrival then the streptavidin-coated plate, and the (contents of the) ziplock bag containing the wash concentrate 20X solution, the TMB substrate solution and the stop solution should be placed at 2-8°C, the plate sealers should be placed at room temperature, and the remainder of the kit stored at -20°C or cooler. The kit is stable for one year under these conditions.

Once opened, the microwell strips of the streptavidin-coated plates may be stored at 2-8°C until the expiration date on the label, but any unused strips should be returned to their foil storage pouch along with the sachet of desiccant included in it and sealed with adhesive tape.

Following the addition of BME to the reaction buffer the kit is stable for one week at 2-8°C. The entire kit is stable for one week at 2-8°C.

REAGENTS AND EQUIPMENT SUPPLIED BY THE USER

- Pipettors (multichannel pipettors desirable) with sterile tips
- Disposable gloves
- 14.5M β-mercaptoethanol (BME)
- Paper towels
- Sterile distilled (deionized) water
- A 37°C incubator
- A 37°C water bath
- A 2-8°C refrigerator
- A 96-well plate reader capable of reading at 450 nm

NOTES BEFORE STARTING

General Comments

Please carefully review the protocol before beginning. Small deviations in the protocol may lead to large discrepancies in the final results observed. All the incubation steps should be performed within +/- 2 min of the indicated times.

Each lot of Express 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 (deionized) water to make a 1X wash buffer solution before use.

Reaction Buffer

The reaction buffer contains both manganese and magnesium, and requires the addition of 0.4 μL β -mercaptoethanol (BME)/mL reaction buffer before use. This solution is stable for one week at 2-8°C following the addition of BME, so unless you are planning to run the entire plate in this timeframe do not add the entire 80 μL BME to the bottle at once. Also, the reaction buffer bottle should always be swirled gently before use.

DS and TS Oligos

The DS and TS oligos are provided as 100X solutions that should be diluted 100-fold into reaction buffer before use. The addition of BME to this reaction buffer that is used as a diluent for the oligos is not required, however its presence does not hinder the reaction. The diluted 1X DS and TS oligo solutions are not stable and the amounts required for an experiment should be prepared just before use. The 100X stocks of these oligos are virtually unaffected by multiple freeze-thaw cycles. In addition the TS oligo is light-sensitive and may leech onto polypropylene tubes. We recommend the TS oligo not be aliquoted into other containers.

HIV-1 Integrase Protein Stability

The HIV-1 integrase protein provided is very stable and its enzymatic activity is completely unaffected by storage at 2-8°C for seven weeks or by five freeze-thawing cycles. We suggest the integrase protein should be transferred to a -20°C freezer or colder until required.

Plate Washing Steps

All liquid should be completely removed from the plate wells for each washing step by patting the plate down onto a stack of paper towels each time that the plate is washed.

Replicates

We recommend all samples and controls be tested using a minimum of three replicates 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

Blocking

1. Prewarm reagents

Place the reaction buffer, the blocking solution, and the wash solution in a 37°C water bath for ≥ 45 min before starting. Prewarm all the other components of the kit except the HIV-1 integrase enzyme by placing them at room temperature for ≥ 45 min.

2. Coat with DS oligo

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 oligo 100X solution 100-fold in reaction buffer (10 μL DS oligo 100X solution and 990 μL reaction buffer per mL). Add 100 μL 1X DS oligo solution per well, seal the plate with a plate sealer and incubate for 1 hr in a 37°C incubator. Return the reaction buffer to the 37°C water bath.

3. Block

Aspirate the liquid from the plate wells and wash three times with 200 μL wash buffer. Add 250 μL of blocking buffer per well, seal the plate with a plate sealer and incubate for 1 hr in a 37°C incubator.

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

Reaction

4. Load the integrase

Thaw the HIV-1 integrase on wet ice or at 2-8°C (~10-15 min) before it is needed and centrifuge the tube briefly (ex. 10,000 RPM X 5 sec) before use. Dilute the enzyme 1:50 in reaction buffer (2 μL HIV-1 integrase and 98 μL reaction buffer per well). Aspirate the liquid from the plate wells and wash three times with 200 μL reaction buffer. Add 100 μL of reaction buffer or integrase solution per well, seal the plate with a plate sealer and incubate for 30 min in a 37°C incubator. Be sure to include reaction buffer alone replicates (without integrase) as a no enzyme negative control. Return the reaction buffer to the 37°C water bath.

5. Add test articles

Prepare test articles by diluting to 2X final desired test concentration in reaction buffer. For example, prepare a 20 μM or 20 $\mu\text{g}/\text{mL}$ solution and serially dilute in reaction buffer when a 10 μM or 10 $\mu\text{g}/\text{mL}$ high-test concentration is desired for use in the assay.

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 and incubate for 5 min at room temperature. Test articles may contain up to 10% dimethyl sulfoxide (DMSO), if desired. The integrase reaction is only marginally affected by the presence of up to 5% DMSO in the final reaction. Be sure to include reaction buffer control replicates (no test article) in each experiment.

6. Add TS oligo

Dilute the required amount of TS oligo 100X solution 100-fold in reaction buffer (10 μL TS oligo 100X solution and 990 μL reaction buffer per mL). Add 50 μL of the 1X TS oligo solution per well directly to the 50 μL buffer/test articles already present in the wells. Seal the plate with a plate sealer and incubate for 30 min at 37°C.

Detection

7. Add HRP antibody

Aspirate the liquid from the plate wells and wash three times with 200 μ L wash solution. Add 100 μ L HRP antibody solution per well, seal the plate with a plate sealer and incubate 1 hr at room temperature.

8. Add TMB peroxidase substrate

Aspirate the liquid from the plate wells and wash three times with 200 μ L wash solution. Add 100 μ L TMB peroxidase substrate per well, seal the plate with a plate sealer and incubate for 1 hr at room temperature.

9. Add TMB stop

Add 100 μ L TMB stop solution directly to the wells containing the TMB substrate. Burst any large bubbles by using a pipette tip. Plates may be sealed with a plate sealer (not supplied) or read directly. 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.

DATA ANALYSIS AND ASSAY PERFORMANCE

Experimental data are shown below to demonstrate how the data derived from the use of this kit should be analyzed and to show how the assay performs. The experiment was set up as shown below using six replicates each of no integrase controls (Blank) and six replicates each of integrase-alone controls (Integrase). Five half-log dilutions of 20% sodium azide (NaN₃) were made in reaction buffer. Note that 50 μ L 20% NaN₃ provides 10% NaN₃ in the 100 μ L strand transfer reaction. Three replicates each of this NaN₃ dilution series were examined.

The following plate layout was used:

Blank	Blank	Blank	10% NaN ₃	10% NaN ₃	10% NaN ₃
Integrase	Integrase	Integrase	3.2% NaN ₃	3.2% NaN ₃	3.2% NaN ₃
Integrase	Integrase	Integrase	1.0% NaN ₃	1.0% NaN ₃	1.0% NaN ₃
Integrase	Integrase	Integrase	0.32% NaN ₃	0.32% NaN ₃	0.32% NaN ₃
Blank	Blank	Blank	0.1% NaN ₃	0.1% NaN ₃	0.1% NaN ₃
			0.1% NaN ₃	0.1% NaN ₃	0.1% NaN ₃

The following data were generated:

0.115	0.146	0.130	0.145	0.141	0.157
1.447	1.669	1.256	0.160	0.242	0.215
1.373	1.503	1.809	0.389	0.514	0.413
0.116	0.114	0.108	1.068	1.139	1.091
			1.591	1.610	1.882

To analyze the data, first determine the mean Blank absorbance in the assay (= 0.122 in this example). Subtract this background absorbance from the other readings:

			0.024	0.020	0.036
1.326	1.548	1.135	0.039	0.121	0.094
1.252	1.382	1.688	0.268	0.393	0.292
			0.947	1.018	0.970
			1.470	1.489	1.761

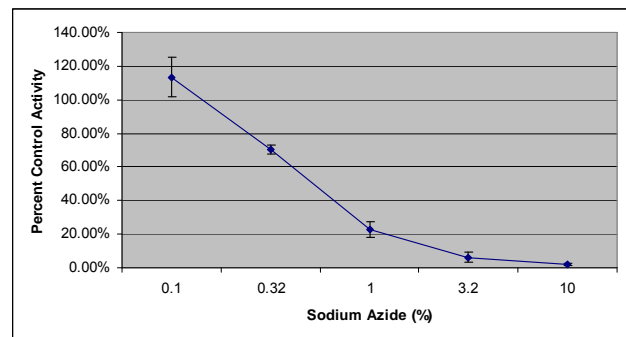
Next determine the mean \pm standard deviation (SD) and CV (= SD/mean X 100%) for the background corrected absorbance for integrase alone replicates and for the test articles. For this example, the results are:

Integrase alone =	1.39 \pm 0.201	CV = 14.5%
10% sodium azide =	0.0262 \pm 0.00833	CV = 31.8%
3.2% sodium azide =	0.0842 \pm 0.0418	CV = 49.7%
1% sodium azide =	0.317 \pm 0.0664	CV = 20.9%
0.32% sodium azide =	0.978 \pm 0.0362	CV = 3.70%
0.1% sodium azide =	1.57 \pm 0.163	CV = 10.4%

Convert 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. For this example, the results are:

Integrase alone =	100% \pm 14.5%
10% sodium azide =	1.89% \pm 0.600%
3.2% sodium azide =	6.06% \pm 3.01%
1% sodium azide =	22.9% \pm 4.78%
0.32% sodium azide =	70.4% \pm 2.61%
0.1% sodium azide =	113% \pm 11.7%

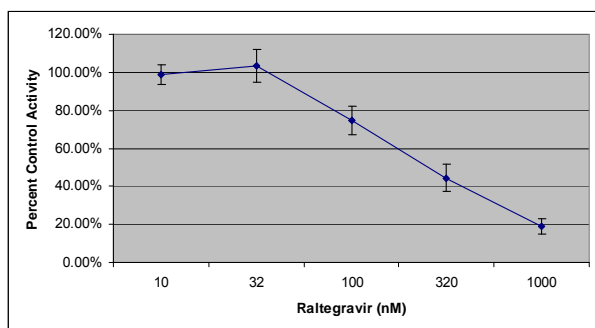
Prepare plots of percent background corrected integrase control versus drug concentration for the test articles as shown below. These plots can be constructed with or without the 0 drug concentration = integrase alone value:



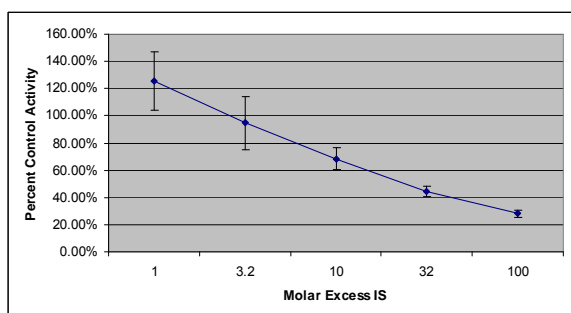
The inhibitory concentration of drug that reduces integrase activity by 50% (IC₅₀) or 90% (IC₉₀) may be interpolated from the curve manually or using more sophisticated programming. In this example, sodium azide showed an IC₅₀ = 0.52% and an IC₉₀ = 2.4%.

The HIV-1 integrase inhibitor raltegravir was found to have an IC₅₀ = 260 nM in the Xpress HIV Integrase Assay. The highest concentration of raltegravir employed did not reduce

control HIV-1 integrase activity by 90% and an IC₉₀ value was not reached.

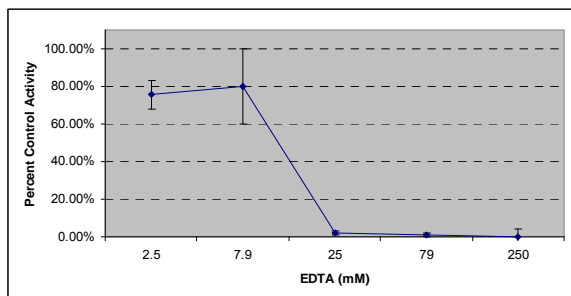


When a TS analogue (inhibitory substrate; IS) that is identical to TS except it does not contain the 3' end modification recognized by the anti-HRP antibody was examined in the assay, the following inhibition curve was observed:



IS showed an IC₅₀ = 24 meaning that a 24-fold excess of this double-stranded oligo inhibited integrase activity by 50%. An IC₉₀ value was not reached.

The concentration of metal ions in the assay buffer is critical for assay performance and the use of chelators are contraindicated in this assay. When HIV-1 integrase activity was examined using EDTA the following was observed:



In this assay EDTA showed an IC₅₀ = 12 mM and an IC₉₀ = 22 mM. These experiments were performed as described above with three replicates at each concentration. Notice how the SD of the 7.9 mM EDTA data point is exaggerated due to the one spurious value observed during the experiment (individual values not shown), which was likely due to our not pipetting integrase into this assay well. When critical results are desired, the use of greater than three replicates per point examined is recommended.

A summary of the absorbance values observed for background-corrected integrase-alone (integrase) and for the IC₅₀ and IC₉₀ values for sodium azide (NaN₃) observed in three independently-performed experiments are shown below.

Expt	Integrase Alone ^a	IC ₅₀ (% NaN ₃)	IC ₉₀ (%NaN ₃)
1	1.3 +/- 0.16	0.52	2.42
2	1.8 +/- 0.29	0.49	1.86
3	2.3 +/- 0.91	0.59	2.03

All 1.8 +/- 0.50 0.53 +/- 0.051 2.1 +/- 0.29

^aMean +/- SD background-corrected absorbance of six integrase alone control wells.

In the third experiment the background-corrected absorbance for integrase alone was greater than two. Depending on the type of plate reader used (i.e., maximum obtainable absorbance value), this type of data may or may not be useable. It is recommended that absorbance values greater than two not be used unless the linearity of the data is validated through the use of a standard curve.

Note: The absorbance values generated by integrase alone vary considerably between experiments, but the IC₅₀ and IC₉₀ values are highly reproducible with CVs = 9.6% and 14%, respectively.

TROUBLESHOOTING GUIDE

Problem	Suggestion
Integrase alone signal >2.0	Reduce incubation times 2 min per step. Or dilute TS with 1 part reaction buffer to 9 parts TS solution.
Integrase alone signal <0.5	Spin down integrase before use. Avoid multiple freeze-thaw cycles.
Wells are stained blue	Add stop reagent before reading plates.
Background is high >0.35	Replace reaction buffer. Use reaction buffer within one week of adding BME and be sure to swirl the bottle before use.

EXPERIENCED USERS PROTOCOL

1. Prewarm reagents, 100 µL DS, 1 hr at 37°C.
2. 3 X 200 µL wash buffer, 250 µL block, 1 hr at 37°C.
3. 3 X 200 µL reaction buffer, 100 µL of 20 µL integrase/mL reaction buffer, 30 min at 37°C.
4. 3 X 200 µL reaction buffer, 50 µL test article in reaction buffer, 5 min at room temperature.
5. 50 µL of TS, 30 min at 37°C.
6. 3 X 200 µL wash buffer, 100 µL HRP antibody, 1 hr at room temperature.
7. 3 X 200 µL wash buffer, 100 µL/well of TMB substrate, 1 hr at room temperature.
8. 100 µL TMB stop solution, read A450.

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.

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