

R&D, Administration and Europe Office

Cisbio Bioassays
 Phone: +33 (0)4 66 79 67 05
 Fax: +33 (0)4 66 79 19 20
 E-mail: bioassays@cisbio.com

Japan Office

Sceti Medical Labo K.K.
 Phone: +81 (0)3 5510 2932
 Fax: +81 (0)3 5510 0130
 E-mail: reagent@scetimedilabo.co.jp

USA Office

Cisbio US, Inc.
 Phone : 888 963 4567
 Fax : 781 687 1500
 E-mail : htrfinfo@cisbio.us



www.htrf.com

HTRF® package insert

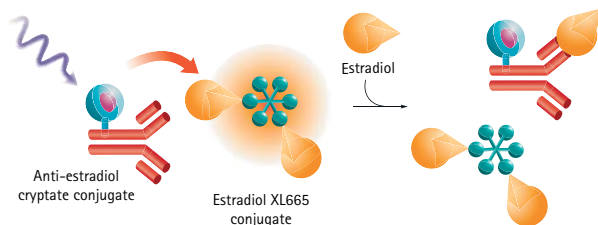
Document reference : 62ESTPEB rev03 (July 2008)

For in vitro research use only
 Storage temperature : 2-8°C

Packaging details :

	96 half-well plate (100 µl)	384 low-volume plate (20 µl)
62ESTPEB	200 tests	1,000 tests

1. Assay description and intended use



This kit is intended for the direct quantitative determination of estradiol in buffered solution or in cell culture supernatants. Its principle is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). The method is a competitive immunoassay in which native estradiol produced by cells and XL665-labeled estradiol, compete for binding to an anti-estradiol MAbs labeled with Cryptate. The specific signal (i.e. energy transfer) is inversely proportional to the concentration of estradiol in the calibrator or in the sample.

The estradiol assay can be run under the following protocol: the “supernatant” protocol (see §3), a standard estradiol assessment in cell supernatant: i) cells are stimulated in a regular cell culture plate, and ii) cell supernatant is then transferred to the assay plate.

2. Background

Estradiol (17 β-estradiol) is a C18 steroid hormone of MW 272.4. It is produced by the graffian follicle of the female ovary, by the placenta during pregnancy and, to a lesser extent, by the adrenal cortices and the male testes.

Estradiol is secreted into the blood stream where virtually all of it is protein bound either to binding globulin or albumin. Estrogenic activity is accomplished via the estradiol receptor which, trigger the response at the nuclear level and the end of the pathway. Target organs include the follicles, uterus, breast, vagina, hypothalamus and the pituitary.

Most breast cancers (about 95%), whether in pre or post menopausal women, are initially hormone dependent, where estradiol plays a crucial role in their development and progression. The hormone and Estrogen receptor complex can mediate the activation of several proto-oncogenes. Consequently, processes that modulate the intracellular concentrations of active estrogens can have the ability to affect the etiology of this disease.

3. Supernatant assay protocol

The supernatant assay protocol must be run in two distinct microplates : i) a culture plate for cell stimulation and ii) an assay plate in which estradiol detection is carried out.

3.1. Reagents supplied

Allow the reagents to come to room temperature for at least 30 minutes before reconstitution.

Supplied reagents	Reagent reconstitution
anti Estradiol-Cryptate** 1 vial, lyophilized	Add 5 mL of reconstitution buffer to each vial. Mix gently.
Estradiol-XL665 1 vial, lyophilized	
Estradiol calibrator. Concentrated estradiol. 1 vial, lyophilized*	See label indications for reconstitution volume. Mix gently after reconstitution.
Reconstitution buffer 50 mM Phosphate buffer, pH 7.0, 0.8M KF 1 vial, see volume on label	
Diluent 50 mM Phosphate buffer, pH 7.0, 0.2% BSA, 0.02% NaN ₃ , preservatives	

* All reagents were lyophilized in 50 mM phosphate buffer, pH 7.0, containing protease free BSA and stabilizers.

** The Cryptate conjugate concentration was optimized for a maximum assay sensitivity and to ensure an average counting of 40,000 cps at 620 nm (384-well low volume format), using the reference PHERAstar Plus reader (BMG LABTECH).

Precaution: HTRF® reagent concentrations have been set for optimal assay performances. Note that any dilution or improper use of the XL665 and Cryptate-conjugates will impair the assay's quality.

3.2. Reagent storage and stability

All reagents should be stored at 2-8°C until reconstituted. Under proper storage conditions, they are stable until the expiry date indicated on the labels.

Reconstituted reagents are stable for two days at 4°C. They can be refrozen (at -80°C) and thawed once.

3.3. Calibration curve preparation

Reconstitute the maximum calibrator according to the indications printed on the label and follow the dilution sequence shown in the following table to draw up the calibration curve. Dilution must be carried out with the diluent (or with freshly made PO4 50 mM, BSA 0.2% pH 7.0).

Calibrator	Calibrator concentration in ng/ml	Preparation
Cal 6	20	100 µL reconstituted calibrator + 400 µL diluent
Cal 5	5	100 µL Cal 6 + 300 µL diluent
Cal 4	1.25	100 µL Cal 5 + 300 µL diluent
Cal 3	0.312	100 µL Cal 4 + 300 µL diluent
Cal 2	0.078	100 µL Cal 3 + 300 µL diluent
Cal 1	0.019	100 µL Cal 2 + 300 µL diluent
Cal 0	0	300 µL diluent

3.4. Sample preparation

Dilute all samples to be assayed with the diluent (or with freshly made PO4 50 mM, BSA 0.2% pH 7.0). Consecutive dilutions should be made within the 0-20 ng/ml range (working solution).

3.5. Assay protocol for 384-well low volume plate (20 µL)

Dispense the reagents in the following order :

- 10 µL standard or sample *
- 5 µL estradiol-XL665
- 5 µL anti-estradiol Cryptate

* For negative control, replace the first reagent by 10 µL of diluent and estradiol-XL665 by 5 µL of reconstitution buffer.

* For positive control, replace the standard by 10 µL of diluent.

- Cover the plate with a plate sealer and incubate for 2 hours at room temperature.
- Remove the plate sealer and read on a compatible HTRF® reader (more information about compatible reader at htrf-assays.com/readers).

3.6. Data reduction and example of a calibration curve

This data should not be substituted for that obtained in the laboratory.

	A(665nm)	A(620nm)	Ratio (1)	Mean Ratio (2)	CV % (3)	Delta F % (4)
Negative control	1,504 1,547	41,507 41,790	362 370	366 (Ratio _{neg})	1.5	
[Calibrator] (ng/mL)						
0	24,443 23,364	44,803 42,593	5,456 5,485	5,471	0.4	1395
0.019	21,055 22,237	39,879 42,608	5,280 5,219	5,250	0.8	1334
0.078	21,746 21,209	42,129 42,052	5,162 5,044	5,103	1.6	1294
0.312	17,140 17,239	40,226 41,141	4,261 4,190	4,226	1.2	1055
1.25	10,183 9,940	40,239 40,975	2,531 2,426	2,479	3	577
5	4,840 4,639	44,828 43,507	1,080 1,066	1,073	0.9	193
20	2,346 2,497	40,738 45,912	576 544	560	4	53

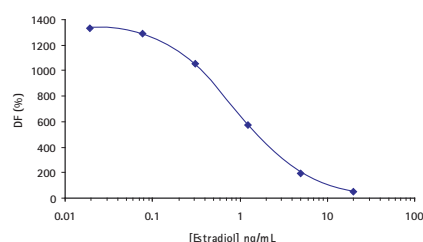
$$1. \text{Ratio} = \frac{A_{665\text{nm}}}{B_{620\text{nm}}} \times 10^4$$

$$2. \text{Mean Ratio} = \frac{\sum \text{ratios}}{2}$$

$$3. \text{CV} = \frac{\text{Std deviation}}{\text{Mean ratio}} \times 100$$

$$4. \text{Delta F} = \frac{\text{Calibrator or sample Ratio} - \text{Ratio}_{\text{neg}}}{\text{Ratio}_{\text{neg}}} \times 100$$

(Ratio_{neg} = negative control)



3.7. Detection limit and conversion to nmol/L

The minimum detectable dose of estradiol is 0.019 ng/ml (dose of mean zero - 2SD). Estradiol concentrations in ng/mL can easily be converted to nmol/l using the following formula :
 $1 \text{ ng/mL} = 3.67 \text{ nmol/L}$.

3.8. Cross-reactivity

	Cross-reactivity (%)
Estradiol	100
Estradiol 3 glucuronide	58.1
Estradiol 3 sulfate	12.3
17 α Ethynylestradiol	0.65
Estrone	0.4
Estriol	0.3
Testosterone	0.2
Danazol	0.02
Tamoxifen	0.007
17 α Estradiol	0.006
Progesterone	0.002
Estrone 3 sulfate	0.002
Corticosterone	<0.001
Cortisone	<0.001
Cortisol	<0.001

3.9 Assay flexibility and miniaturization

When used as suggested, the kit will provide sufficient reagents for 1,000 tests using using a 384- well low volume plate in 20 μL final assay volume (HTRF® packaged basis).

To change to other plate formats (96 half-well or 1536-well) and final volumes (100 μL to less than 10 μL), the volume of each assay component is simply proportionally adjusted in order to maintain the reagent concentrations as for the 20 μL final assay volume. For instance, in the case of the 1536-well format in 10 μL final volume, half as much material per well is used, thereby allowing 2,000 tests to be run. The performances of the HTRF® assay remain the same whatever the level of miniaturization.

Assay components	Volume proportion	Assay format		
		1536-well(10 μL)	384-well low volume(20 μL)	96 half-well(100 μL)
Sample	2 volumes	5 μL	10 μL	50 μL
XL665 conjugate	1 volume	2.5 μL	5 μL	25 μL
Cryptate conjugate	1 volume	2.5 μL	5 μL	25 μL
		2,000 tests	1,000 tests	200 tests

Plate references : 96 half-well plate (Costar # 3694 or equivalent), 384-well low-volume plate (Greiner # 784076), 1536-well (Greiner # 782086).