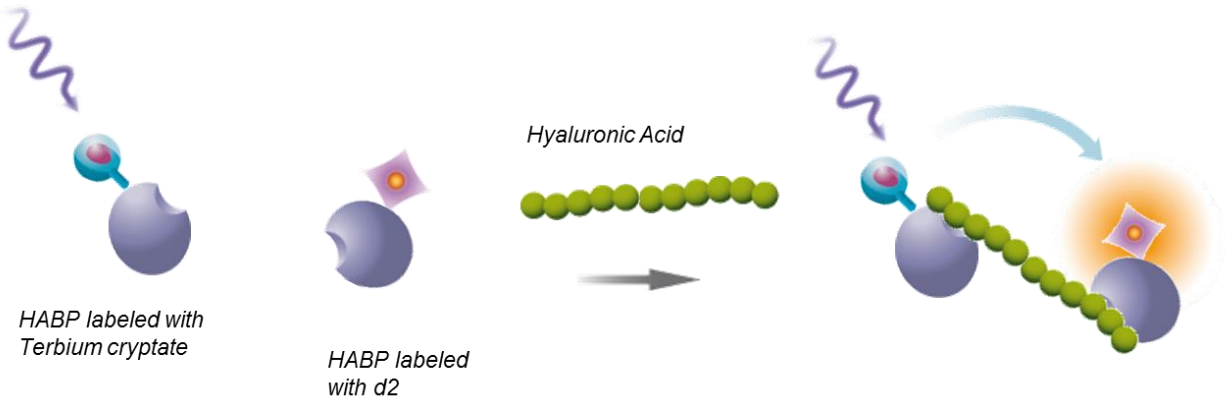
HTRF® Hyaluronic Acid (HA) Assay
500 tests

Section 1

Assay Description:

This kit is intended for the determination of levels of Hyaluronic Acid (HA) in cell supernatant. The principle of this kit is based on HTRF technology (Homogeneous Time-Resolved Fluorescence). As shown in Figure 1, HA is detected in a sandwich HTRF assay using Hyaluronic Acid binding protein (HABP), one labeled with an Terbium-cryptate donor and the other with a d2 acceptor dye. The specific FRET signal is directly proportional to the amount of HA in the sample.

Figure 1: **Diagram of the HTRF HA detection assay**



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Section 2

Reagent Description

Components	Quantity / Storage	Stock Solutions	Number of Tests	Volume
HABP-d2*	1 vial / Frozen	50X	500	50uL
HABP-Tb*	1 vial / Frozen	50X	500	50uL
HA Standard*	1 vial/ Frozen	500 ug/mL		100uL
Dilution Buffer	1 bottle / Frozen or 4°C	1X		20mL
Binding Buffer	1 bottle / Frozen or 4°C	4X		1 x 1.5mL
Detection Buffer	1 bottle / Frozen or 4°C	1X		6mL

Reagent Handling

*Store labeled proteins at -80°C.
Make aliquots of labeled proteins and store at -80°C, to avoid numerous freeze/thaw cycles.

Section 3

Working Solution Preparation

Solution A
HABP-d2
Make a 50 fold dilution.
For example, add 2450uL of *detection buffer* to 50uL of HABP-d2 stock solution.

Solution B
HABP-Tb
Make a 50 fold dilution.
For example, add 2450uL of *detection buffer* to 50uL of HABP-Tb stock solution.

Standard Dilutions

Using HA Standard stock solution (500ug/mL), prepare the following dilutions:

Dilution 1: Prepare a 10-fold dilution using stock solution.
For example: add 50uL of stock solution to 450uL of Dilution Buffer (50ug/mL).

Then prepare the following serial dilutions:

Table 1. HA Standard Curve Dilutions

Standard #	uL of Diluent	uL of Standard#	Standard Concentration (ng/mL)
1	980	20 of **Dilution 1**	1000
2	200	200 of #1	500
3	200	200 of #2	250
4	200	200 of #3	125
5	200	200 of #4	62.5
6	200	200 of #5	31.25
7	200	200 0f #6	15.62
8	200	200 0f #7	7.8
9	200	200 0f #8	3.9
10	200	0	0

Standard #10 is the assay negative control.

Binding Buffer:

Dilute 2 fold the Binding Buffer stock solution with distilled water (e.g. take 1mL of Binding Buffer stock solution and add it to 1mL of distilled water). Mix gently.

Section 4

Assay protocol for 384-well low volume, WHITE plate (e.g. Greiner part# 784075), 20uL final volume

- Dispense the reagents in the following order:

  5uL of standard or cell supernatant to be tested
  5uL of Binding Buffer 2X
  5uL of HABP-d2 (Solution A)
  5uL of HABP-Tb (Solution B)

- Cover the plate with a plate sealer and incubate overnight at RT or 4°C for better performances

- Remove the plate sealer and read on a compatible HTRF reader (information on HTRF readers at www.htrf.com/readers)

Perform the following assay controls:
Buffer control: To make sure that buffers are not contaminated by cryptate and do not generate any background fluorescence.
Cryptate control: To check the Cryptate signal at 620 nm.
Negative control: Combination of donor and acceptor in the presence of appropriate buffer without sample (HA). This control is used for the calculation of delta F.

Table 2: Reagent dispensing for HA assay and controls

	Buffer control	***Cryptate control***	***Negative control***	***Sample***
Diluent Only	5uL	5uL	5uL	
Cell Supernatant				5uL
Binding Buffer	5uL	5uL	5uL	5uL
Detection Buffer	10uL	5uL		
HABP-d2			5uL	5uL
HABP-Tb		5uL	5uL	5uL

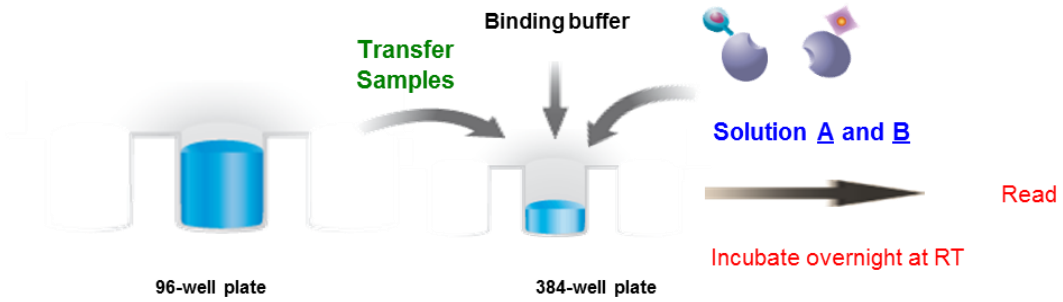


Figure 2. HTRF HA Assay Protocol

Results

The table below is representative of data obtained after incubation overnight at room temperature. The standard curve covers a range of 3.9ng/mL to 1,000ng/mL of HA. Incubation at 4°C will improve assay performances.

Table 3: Typical results after overnight at RT.

HA [ng/mL]	A(665nm)	A(620nm)	Ratio (1)	Delta F% (2)
1000	16921	11548	14655	429%
500	14682	11755	12488	351%
250	11804	12534	9418	240%
125	8903	12290	7245	162%
62.5	6857	12630	5429	96%
31.25	5433	12749	4262	54%
15.6	4637	13026	3560	29%
7.8	4057	12753	3181	15%
3.9	3892	12832	3033	10%
0	3838	13857	2770	0%

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

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

(Ratio_{neg} = negative control)

Ratio: The ratio is calculated for each well individually and multiplied by a factor of 10,000 for easier data processing. The mean and standard deviation are calculated for replicates.

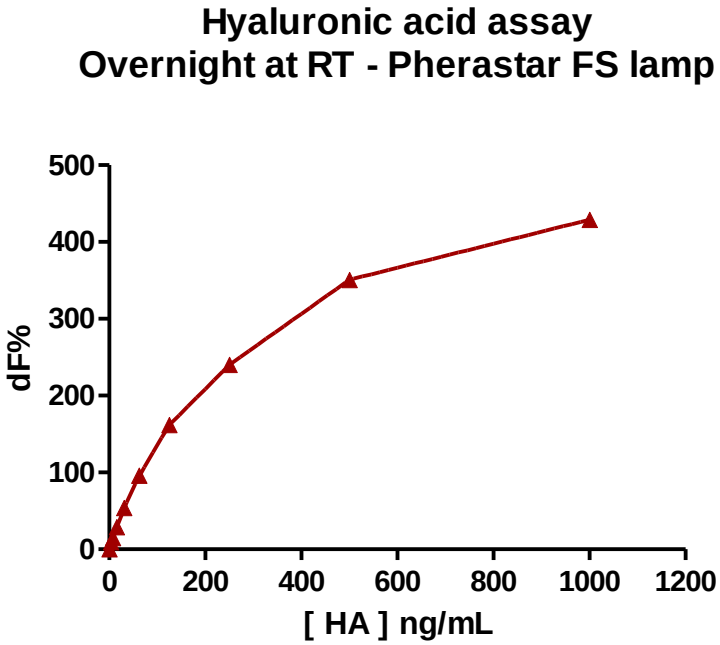
Delta F%: (DF%) is used for the comparison of day to day runs of the same assay. It reflects the signal to background fluorescence of the assay. The negative control is used as the internal assay control.

For more information regarding data reduction, please visit our website:
http://www.htrf.com/htrf-technology/ratio-and-data-reduction

Section 5

Typical results

Standard curve tested following the kit protocol. Reading performed on a PheraStar FS lamp (BMG Labtech).



For complete information regarding data reduction for HTRF assays, please visit our website at:
http://www.htrf.com/htrf-technology/ratio-and-data-reduction