TECHNICAL NOTE

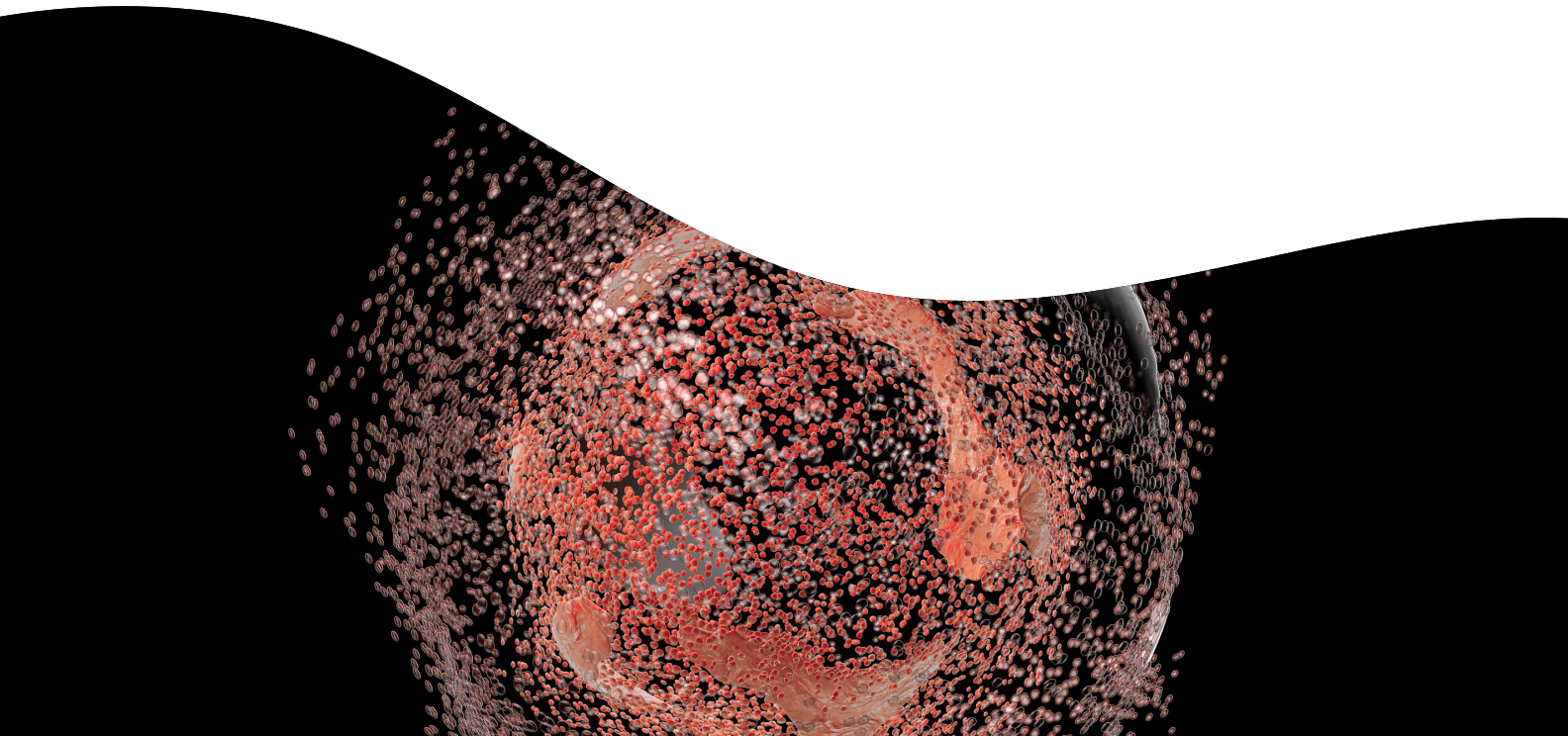
Guidelines for cell culture and lysis in different formats prior to HTRF detection.

This technical note provides seeding and lysing recommendations for a number of cell culture vessels.

Abstract

Cell culture can be carried out in large volume containers, such as T75 flasks, or medium volume containers such as 96-well, 24-well, 12-well or even 6-well plates. Flasks deliver a straightforward, efficient approach to cultivating large quantities of cells when such batches are needed. Cell culture in micro plates is the preferred format when multiple conditions and parameters need to be tested on cells.

This technical note provides guidelines on adapting the standard 96-well plate protocol of seeding and lysing cells to different culture formats such as T75 flasks, or 6-well, 12-well, and 24-well plates. The note demonstrates that culture in different vessels can give similar results if care is taken to control operational parameters, such as lysis volume.



General assay principle

The standard protocol for all HTRF® phospho & total assays consists in growing cells in the chosen container, treating them with the compound of interest, and then lysing them. Lysates are next transferred to Revvity 96-well low volume plate or 384-well small volume white plate before detection.

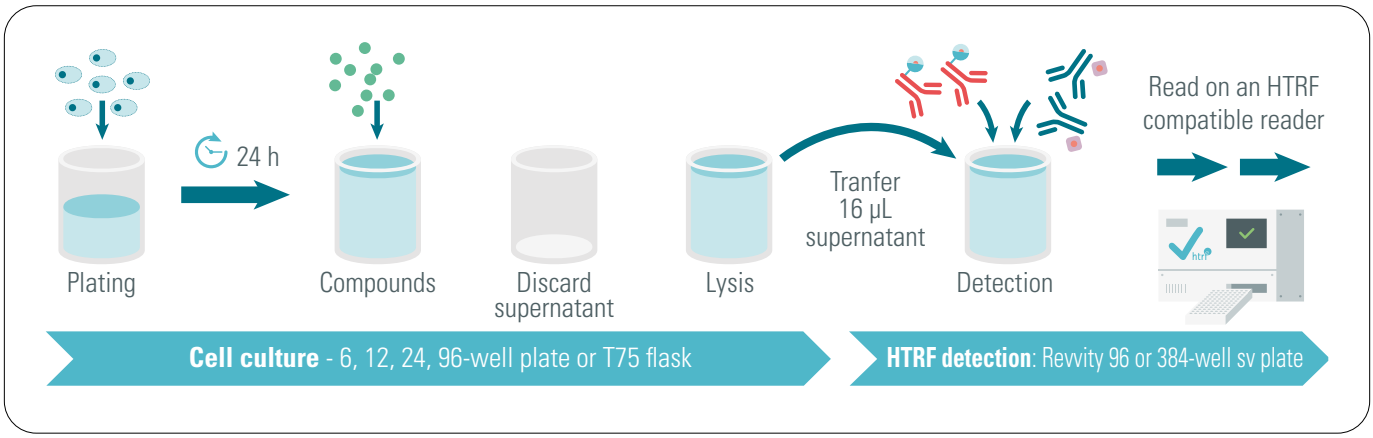


Figure 1: Key steps for handling cells on which to perform an HTRF assay.

Methodology of transfer from cell culture vessel to HTRF detection microplate

Recommendations for seeding numbers and volumes for different culture containers are provided in table 1.

Following seeding, cells are incubated 24 h at 37°C, 5% CO_2. After cell treatment, the culture medium is removed and replaced with the indicated volume of lysis buffer for 30 min.

Once the cells are lysed, 16 µL of cell lysate are transferred to a 384-well or Revvity 96-well HTRF detection plate, and 4 µL of pre-mixed HTRF antibodies are added to each well.

Table 1 shows key features such as optimal cell density, medium volume and lysis volume, depending on the microplate or flask used for cell culture. We recommend following these indications in order to optimize the quality of your results and ensure your assays stay within their linearity range. If the amount of lysis buffer provided in the kit is not sufficient for your needs, remember that more can be purchased separately.

Table 1: Cell seeding density, culture medium volume and lysis volume recommended for each cell culture vessel.

Cell culture vessel				Detection microplate		
Microplate	Seeding cell density (10^3 cells/well)	Culture medium (µL)	Lysis buffer (µL)	Microplate	Cellular lysate (µL)	HTRF antibodies (µL)
96w	25-100	50	50	96 low volume HTRF detection plate or 384 w	16	4
24w	140-550	500	250			
12w	290-1150	1000	500			
6w	700-2800	2500	1500			
T75cm2	6000-24000	10000	12000			

Case study: detection of Total-ERK protein (64NRKPEG) in the scaled-up lysates

A431 cells were seeded in microplates (from 6- to 96-well plates) or in a T75 cm2 flask, at 3 different cell densities. After plating and remaining 24 h at 37°C, 5% CO_2, the cells were lysed. The exact experimental conditions are reported in Table 2. Cellular lysates were then transferred to 96-well plates, and analyzed with an HTRF reader.

Table 2: Cell seeding density, Lysis volume and Cell concentration used for each cell culture vessel.

Cell Culture in 96 well plate			
Cell seeding density (cells 10^3/well)	25	50	100
Lysis volume (µL)	50	50	50
Cell concentration (cell/µL)	500	1000	2000

Cell Culture in 6 well plate			
Cell seeding density (cells 10^3/well)	750	1500	3000
Lysis volume (µL)	1500	1500	1500
Cell concentration (cell/µL)	500	1000	1000

Cell Culture in T75 flask			
Cell seeding density (cells 10^3/well)	6000	12000	24000
Lysis volume (µL)	12000	12000	12000
Cell concentration (cell/µL)	500	1000	2000

HTRF results obtained from these cell cultures were read on 96-well low volume HTRF detection plate and are compared in Figure 2.

As shown, different culture vessels can give the same assay result if care is taken to maintain the ratio of cell number to lysis buffer constant.

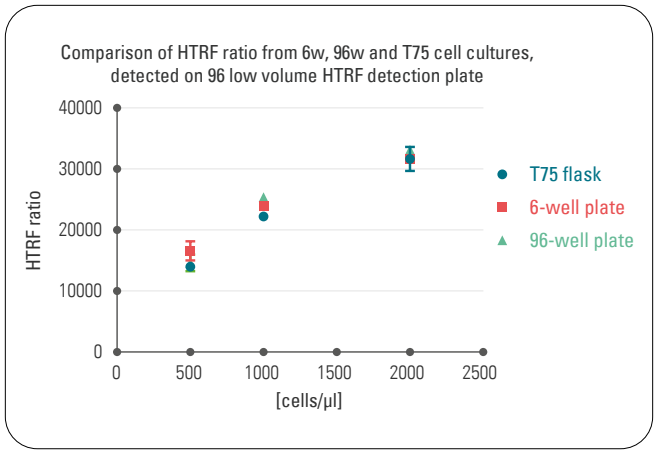


Figure 2: Comparison of HTRF ratio obtained from cells grown in different vessels.

Conclusion

This technical note provides seeding and lysing recommendations for a number of cell culture vessels, and demonstrates that users can expect similar results so long as the ratio of cell numbers per volume of lysis buffer used remains similar.

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