HTRF Alpha-Tubulin Housekeeping kit: Matching versatility with dynamic range for unprecedented performance.

This application note highlights the unmatched versatility of the HTRF Alpha-Tubulin assay kit and provides a convincing demonstration of its applicability to a variety of sample and experimental conditions.

Abstract

Alpha-tubulin is a component of microtubules. Due to its essential role in the cytoskeleton, this protein is ubiquitously and constitutively expressed in every tissue. Moreover, its amino acid sequence is highly conserved and is thus identical in almost all species. These characteristics make it one of the most commonly used housekeeping proteins.

The HTRF® Alpha-Tubulin Housekeeping kit is based on a homogeneous TR-FRET immunoassay format and measures the endogenous level of α-tubulin in cell and tissue lysates.

This application note highlights the unmatched versatility of the HTRF Alpha-Tubulin assay kit (#64ATUBPEG/H) and provides a convincing demonstration of its applicability to a variety of sample and experimental conditions. Compatibility with all Revvity lysis buffers, species cross-reactivity, as well as correlation with BCA Total Protein assay are presented below for the reader's review.

Assay workflow

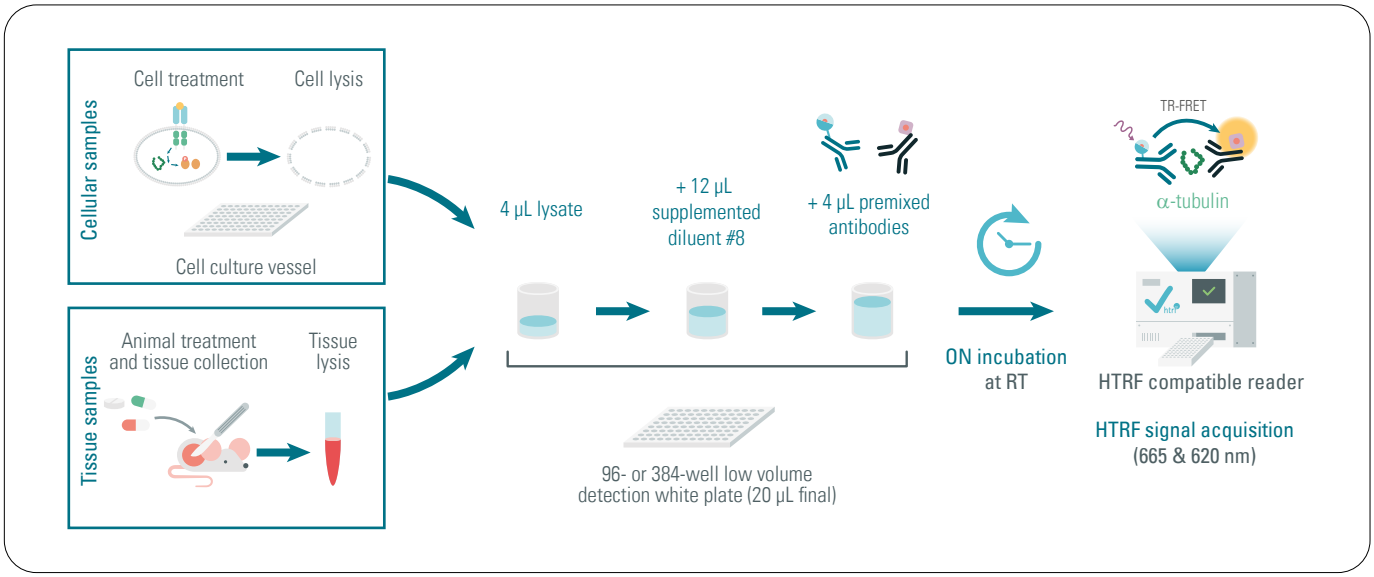

Figure 1: Assay workflow

The assay can be carried out on cell or tissue lysates generated using either the Alpha-Tubulin Housekeeping kit (#64ATUBPEG/H) lysis buffer (#4) or any of the lysis buffers provided in the Revvity phosphorylation assay kits (#1, #2, #3 or #4).

For HTRF detection, 4 µL of lysate are transferred into a low volume white microplate and 12 µL of kit diluent are added before the dispensing of 4 µL of premixed detection antibodies. The HTRF signal is recorded following an overnight incubation at RT.

The low sample volume of 4 µL offers a great advantage when working with precious samples or when limited quantities of lysates are available. It enables a multi-parametric analysis of the housekeeping protein α-tubulin with the phospho- and/or total protein(s) of interest in parallel on the same lysate.

Compatibility with cell signaling lysis buffers

HeLa cells were plated at different cell densities in 96-well culture plates and grown for 24h at 37°C-5% CO2 before lysis with 50 µL of supplemented lysis buffer #1, #2, #3 or #4.

As shown below, the assay is compatible with all cell signaling buffers. The best performance is obtained with lysis buffers #2, #3 and #4. Lysis buffer #1 provides the lowest signal for an otherwise comfortable overall assay window so that even the smallest cell densities can easily be resolved.

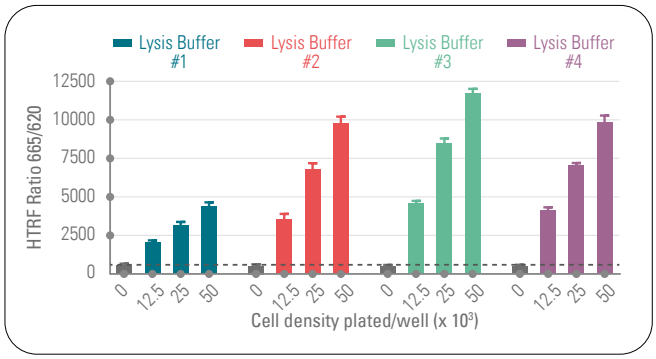

Figure 2: Compatibility with cell signaling lysis buffers

These results demonstrate that the assay can be performed in parallel for every HTRF phospho- or total protein assay on the same lysate. For more information, please refer to the lysis buffer compatibility table: www.Revvity.com/htrf-lysis-buffer-compatibility.

Multi-species cross-reactivity

The HepG2, NIH/3T3 and CHO cell lines were plated at different cell densities in 96-well culture plates and grown for 24h at 37°C-5% CO2 before lysis with 50 µL of supplemented lysis buffer #4.

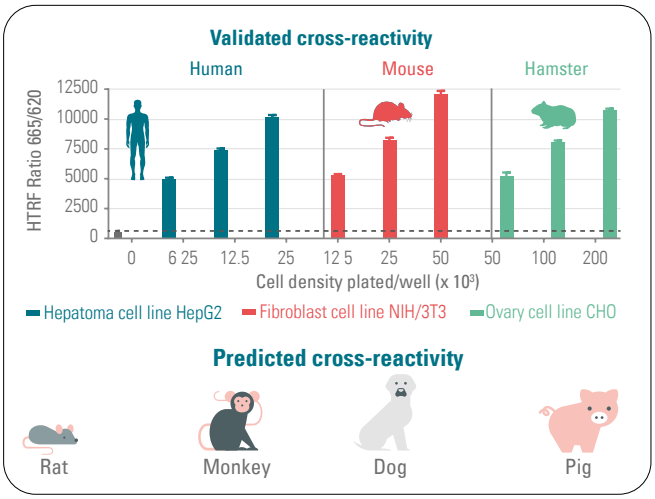

Figure 3: Validated and Predicted cross-reactivity

The assay has been validated on human, mouse and hamster cell lines and is also predicted to work on samples from rat, monkey, dog and pig origins. It is therefore compatible with the most commonly used animal species.

Assay range adapted to frequently used cell types

Various cell types were tested using a wide cell density range. Suspension cell lines and PBMCs isolated from human blood samples were dispensed into half 96-well plates (under 30 µL) and directly lysed with 10 µL of supplemented lysis buffer #4 4X. Adherent cell lines were plated and lysed as described in the previous section. The lysates were directly analyzed (neat), or prediluted in the supplemented lysis buffer 1X when necessary.

Suspension Cells

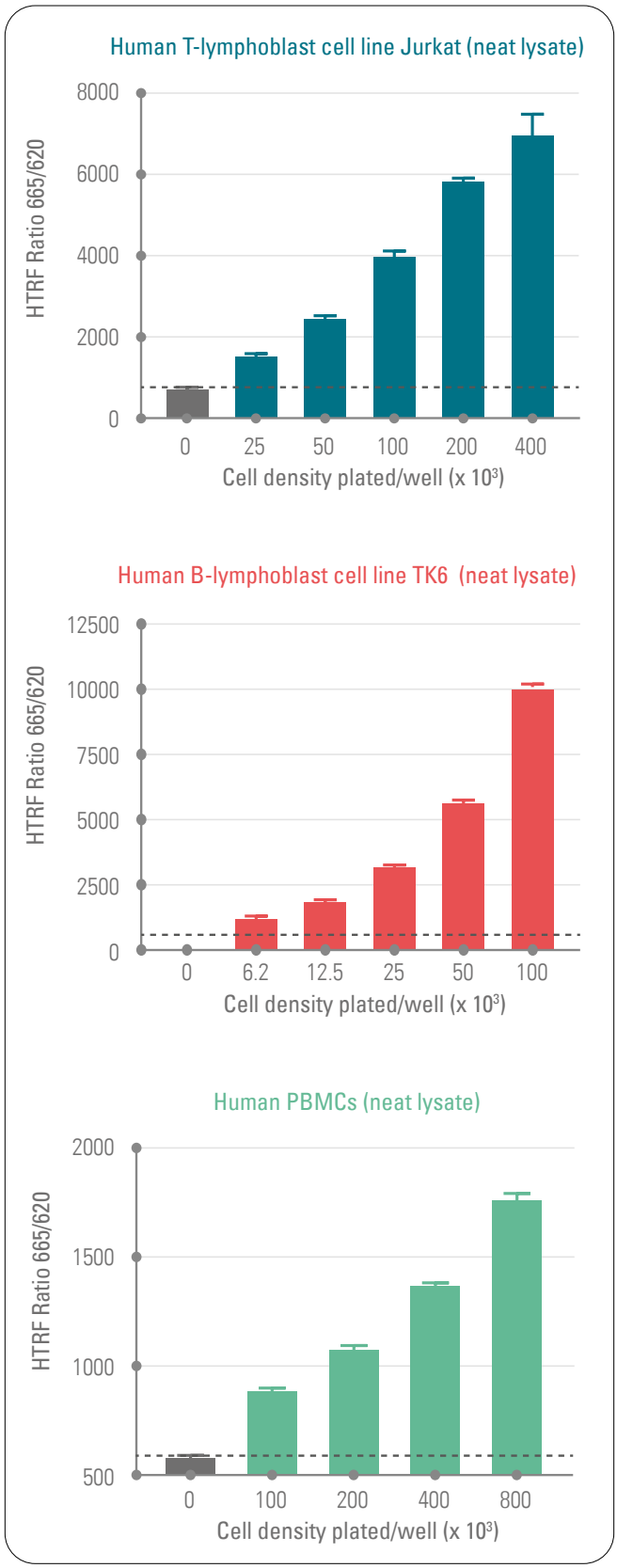

Figure 4: Assay range adapted to suspension cells

Adherent cells

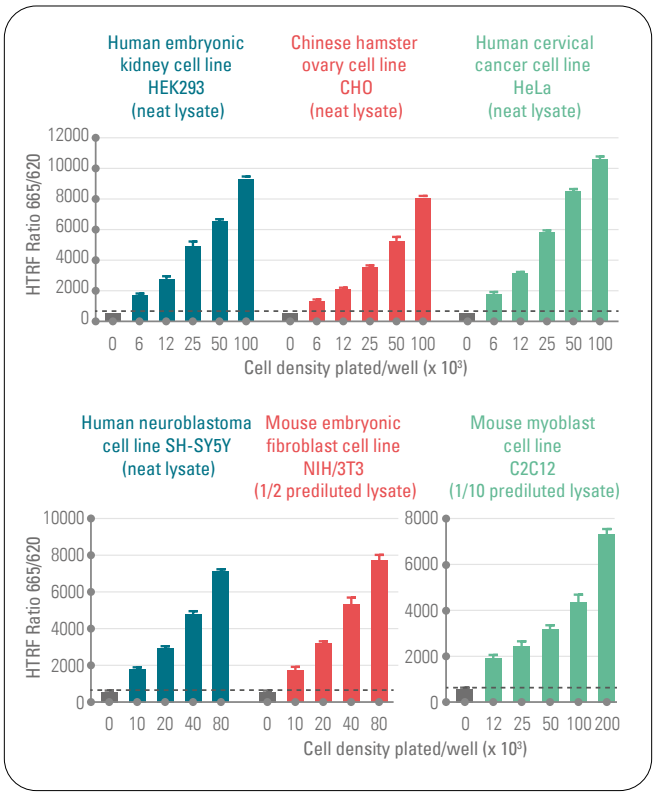

Figure 5: Assay range adapted to adherent cells

As demonstrated by these results, the assay is suitable for working on all types of cellular models: adherent, suspension, immortalized cell lines or primary cells, from different tissue types.

The assay's dynamic range and sensitivity are adapted to commonly tested cell densities, and for most cell types without the need to predilute the lysate. Even where highly expressing cell lines need to be assayed, a 2 fold to 10 fold predilution results in alpha-tubulin concentrations being within the assay range.

Compatibility with tissue samples

Liver tissue lysates from three DIN (Diet-Induced NASH) mice* were prepared with lysis buffer #3 and analyzed as described in the technical note www.Revvity.com/drug-discovery/optimize-your-htrf-cell-signaling-assays-tissues. After total protein quantitation, samples were adjusted to the same concentration and serially diluted in the supplemented lysis buffer 1X.

As shown on the right, the assay sensitivity enables a comfortable detection of α-tubulin in samples containing less than 1 mg/mL of proteins. Moreover, the dynamic is adapted to the analysis of highly concentrated lysates (up to 5 mg/mL of proteins), without predilution of the samples.

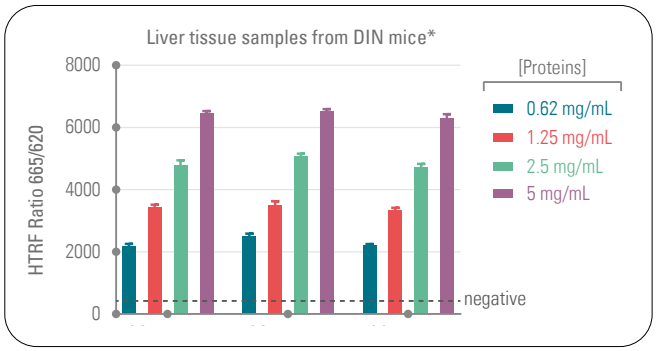

Figure 5: Compatibility with cellular samples

*DIN mouse liver samples were kindly provided by the preclinical CRO Physiogenex (Labège, France).

Correlation with the BCA protein assay

Adherent human and mouse cell lines were plated at different cell densities and lysed as previously described. Serial dilutions of liver tissue lysates from DIN (Diet-Induced NASH) and MCD (Methionine- and -Choline Deficient) mice* were prepared as mentioned in the previous section. The level of the housekeeping protein α-tubulin was measured in these samples using the HTRF assay. In parallel, the protein concentrations were determined from the same lysates using the BCA protein assay (QuantiPro™ BCA Assay Kit, Sigma-Aldrich) according to the manufacturer's instructions. For both methods, lysates were analyzed either neat or prediluted in the appropriate buffer, thus ensuring all samples were tested within the respective assay linear ranges.

Cellular samples

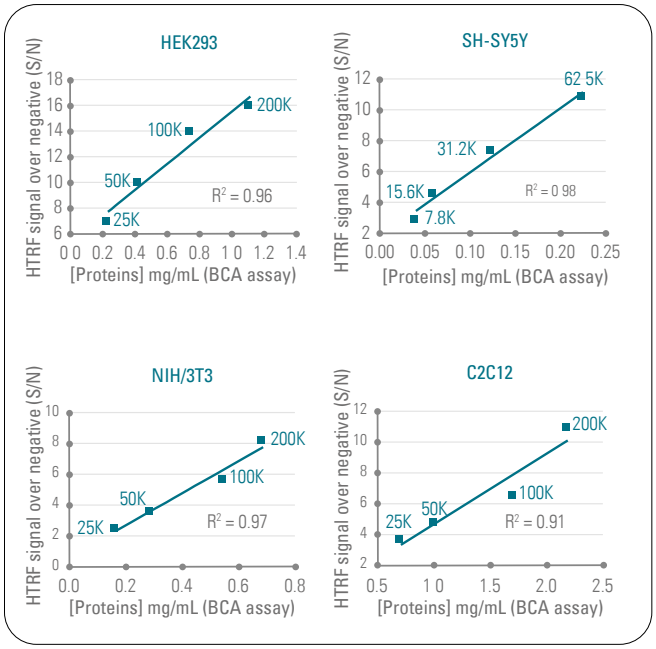

Figure 6: Correlation with the BCA protein assay

Tissue Samples

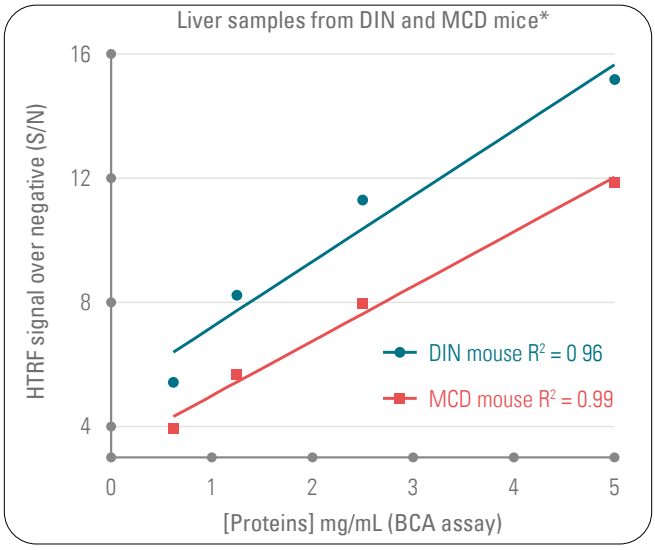

Figure 6: Compatibility with tissue samples

*DIN and MCD mouse liver samples were kindly provided by the preclinical CRO Physiogenex (Labège, France).

Each graph represents the HTRF α-tubulin signal over negative (S/N) versus the protein concentration determined by BCA. The cell densities used for the correlations are mentioned next to each point.

Using human cell lines, mouse cell lines, or tissues, the level of α-tubulin measured by HTRF is always properly correlated to the concentration of proteins determined by the BCA method (with R2 > 0.91).

These results demonstrate that α-tubulin is an appropriate housekeeping protein suitable for checking the effect of compounds on your protein(s) of interest. For more information, please refer to the application note: www.Revvity.com/alpha-tubulin-hk-for-cell-signaling-assays.

Conclusion

This application note highlights the multiple advantages of the HTRF Alpha-Tubulin housekeeping kit, including convenience, adaptability and relevance.

First, the compatibility of the assay with all cell signaling lysis buffers and the low sampling volume enable a multi-parametric analysis of α-tubulin and the phospho-/total protein(s) of interest on the same lysate, which is of great interest when working with precious samples.

Secondly, the assay is compatible with samples from different animal species and with wide-ranging biological complexity (from immortalized cell lines to primary cells and tissues). As such, the assay is applicable to all steps of the drug discovery process, from HTS to preclinical studies. The assay range is adapted to the usual cell densities of adherent and suspension cell models, with no need to predilute the lysate in most cases.

Finally, α-tubulin levels as measured by HTRF were correlated to total protein content, thus demonstrating the reliability and robustness of the assay.

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com