# Multiple therapeutic areas addressed with HTRF KinEASE assays


## Abstract

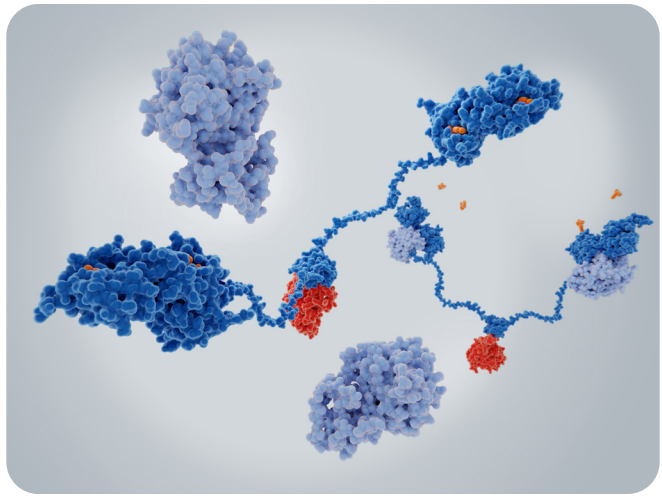
Despite the importance of kinase investigations, only a few have been characterized. Revvity developed special products dedicated to kinase studies called KinEASE.™ In this literature review, three case studies using KinEASE assays are compiled regarding various therapeutic areas.


## Introduction

The human kinome consists of 518 proteins and 20 lipid kinases (Wilson et al., 2018). Even if kinases are categorized in different groups, most of them are highly conserved. Kinase proteins are key players in many cellular processes, including cell proliferation, metabolism, transcription, etc. Owing to their essential role in cell signaling, any dysregulation or mutation in kinases can result in a large number of diseases, such as cancer, neurological disorders, or cardiovascular diseases. Despite this, only 80 kinases have so far been targeted for the treatment of disorders, mainly for cancer.

Revvity has developed a specific product range, the HTRF KinEASE kit series, to investigate kinase activity, characterize them, and screen for inhibitors. The four kits offer a semi-universal method for assessing phosphorylation on Serine/Threonine (3 STK kits) and Tyrosine (1 TK kit) residues, respectively. With this method, over 272 kinases have already been validated.

With HTRF® KinEASE assay kits, numerous compounds have been identified as inhibiting kinase activities, and their mechanism of action has been characterized in different therapeutic areas. In this literature review, some cases of KinEASE product applications in different diseases have been compiled.



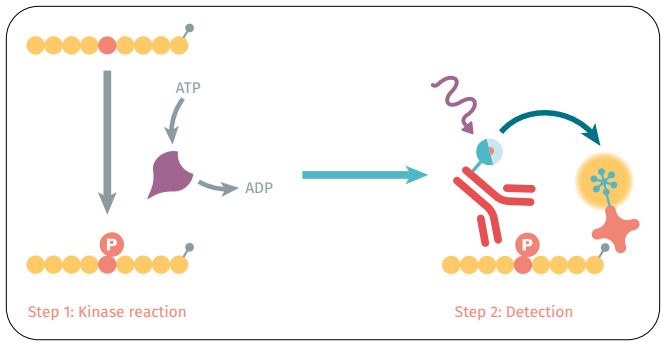



Figure 1: KinEASE assay principle

*The assay involves two steps. Step 1 (kinase reaction) involves incubating the kinase with or without compounds and the appropriate Revvity substrate with ATP and enables the reaction. During step 2 (detection), phosphorylated substrate will be detected on the one hand by the STK-Antibody labeled with Eu3+-cryptate and on the other by the streptavidin XL allowing TR-FRET signal to occur.*

## Cancer studies – Bcr-Abl in chronic Myeloid Leukemia

Chronic myeloid leukemia or CML is a myeloproliferative disorder where two proteins, BCR and Abl, are fused, leading to a constitutive activation of ABL kinase and downstream kinase on several signaling pathways. Most of the inhibitors found have often been invalidated due to cellular resistance during treatment and its insensitivity to ABL mutation, especially the T315I mutation. In 2017, Sun et al found an imidazopyridazine-based compound called CT-721 as an inhibitor of WT BCR-ABL and T315I mutant (Sun et al., 2017). In this study, the HTRF KinEASE-TK kit was used to analyze kinase enzyme activity and CT-721 compound potency.

CT-721 inhibition on the BCR-ABL kinase and T315I mutant BCR-ABL kinase is efficient, with $IC_{50}$ values of 21.3 ± 1.1 nM and 65.0 ± 6.2 nM, respectively (Table 1).

Table 1: Kinase inhibition profile of CT-721 (Sun et al., 2017)

| Kinase | $IC_{50}$ (nM) |
|---|---|
| Abl (WT) | 21.3 ± 1.1 |
| Abl (T315I) | 65.0 ± 6.2 |
| Abl (E225K) | 2.9 ± 0.3 |
| Abl (G250E) | 3.9 ± 0.2 |
| Abl (Y253F) | 2.1 ± 0.2 |
| Abl (H369P) | 3.6 ± 0.7 |
| Abl (M351T) | 3.7 ± 1.4 |
| Abl (Q252H) | 4.3 ± 2.0 |
| c-Kit | 9.2 ± 0.4 |
| VEGFR2 | 48.9 ± 3.9 |
| PDGFB | 106 ± 10 |
| EGFR | 130 ± 20 |
| Alk | >1000 |

Strong inhibition on 6 other imatinib-resistant mutants (E225K, G250E, Y253F, H369P, M351T and Q252H) can also be observed. Moreover, CT-721 specificity has been determined with the inhibition profile over other human kinases involved in the progress of CML disease, including c-Kit, VEGFRF2, PDGFRβ, EGFR and Alk. CT-721 also markedly inhibited c-Kit, VEGFR2, PDGFRβ, and EGFR, implying that CT-721 could exert additional effects on CML other than targeting BCR-ABL kinase.

Furthermore, CT-721 showed dose and time dependent inhibitions on BCR-ABL kinase activity. After Abl pre-incubation with CT-721 for 2 hours, $IC_{50}$ values decreased 9-fold from 12.1 nM to 1.3 nM, meaning that CT-721 inhibited the ABL enzyme in a time-dependent manner.

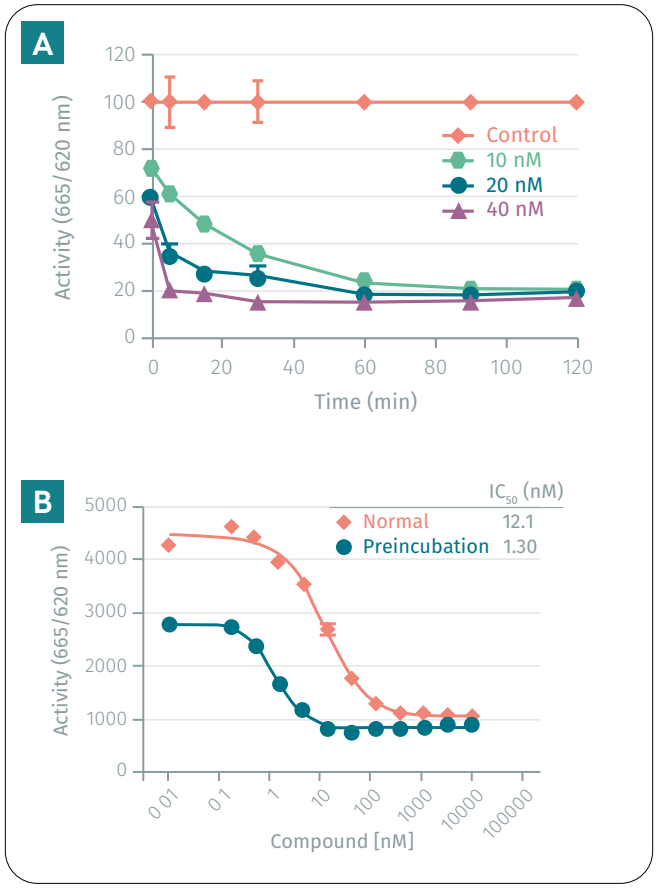



Figure 2: CT-721 inhibition on the BCR-ABL kinase and T315I mutant BCR-ABL kinase. A. Kinase inhibition profile of CT-721. B. Concentration-response analysis of CT-721 at the indicated concentrations (Sun et al., 2017).

This study showed that the CT-721 compound is a strong and time-dependent inhibitor for WT and T315I mutant BCR-ABL kinase, making it a potential application for CML treatment.

## Neuroscience studies – characterization of A1 compound action for chronic pain treatment

The NGF/TrkA pathway plays a central role in the physiological function of chronic pain. Tropomyosin receptor kinase A (TrkA) is a membrane-bound receptor which, upon neurotrophin binding, phosphorylates itself and members

of the MAPK pathway. TrkA inhibitors are therefore much sought-after for chronic pain therapy. Some low molecular weight T1 and T2 TrkA pan-inhibitors have been developed. However, undesirable side effects have been observed for this type of inhibitor, indicating the need to find new, better types. Furuya et al. showed that a selective-TrkA inhibitor called A1 was specific and highly potent on this kinase (Furuya et al., 2017). In that study, the X-ray crystal structure showed that A1 bound to the TrkA juxtamembrane (JM) region. To determine the importance of the JM region on A1 kinase inhibition, a kinase activity assay was performed using the HTRF KinEASE-TK assay, with two types of TrkA kinase domains with (JM+) or without (JM-).

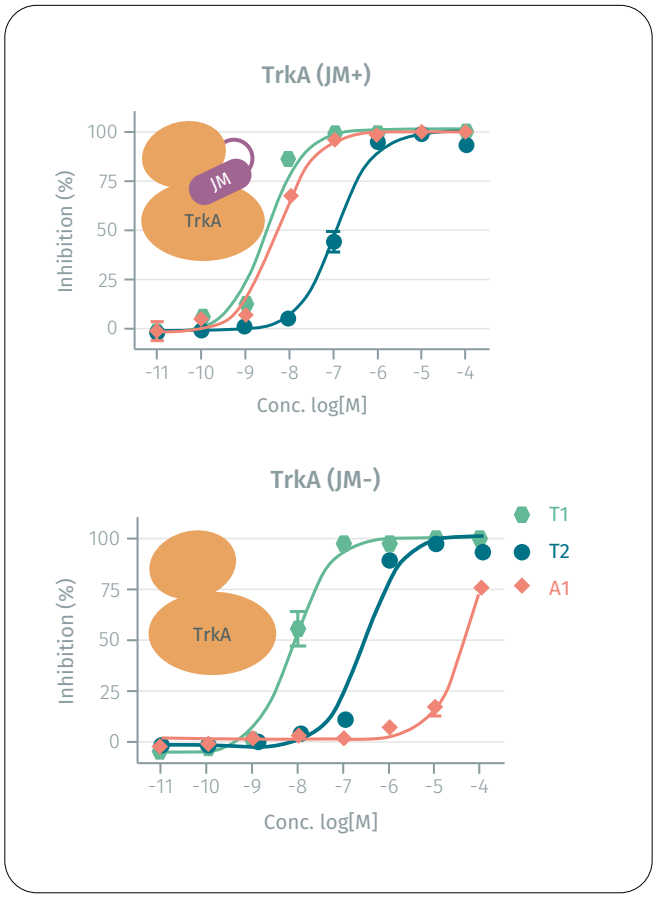



Figure 3: TrkA JM domain importance.

*HTRF KinEASE-TK assay using two types of TrkA kinase domains with or without the JM region (JM+ or JM-, respectively) (Furuya et al., 2017)*

The results shown in Figure 3 demonstrate that T1 and T2 act indifferently to the presence or lack of JM, unlike A1 which has a markedly decreased efficacy without the JM region.

The results suggest that the JM region is determinant for A1 selectivity and affinity. Here, kinase inhibition assay confirmed the importance of the JM region for A1 action on the kinase activity. These results highlighted the action of a new inhibitor which possesses an allosteric binding mode on TrkA kinase.

## Autoimmune disease studies – Dmf action on Rsk2 or Psoriasis treatment

Psoriasis is a long-lasting autoimmune disease characterized by red, itchy, scaly patches of abnormal skin. One of the most prominent treatments used is dimethyl fumarate (DMF). However, DMF has a slow action and generally takes several weeks of high dose administration to be efficient. Despite its widespread use, its action is poorly known, and it seems necessary to elucidate its role more clearly.

Andersen et al. showed that DMF binds to Ribosomal S6 kinase 2 (RSK2), especially to the C-terminal kinase domain (CTKD) (Andersen et al., 2018). To determine DMF's efficacy on RSK2 activity, *in vitro* assays were performed with Revvity's HTRF KinEASE STK1 assay.

The results shown in Figure 4 A demonstrate the inhibitory action of DMF on RSK2, as DMF leads to full inhibition of $RSK2^{CTKD}$ with an $IC_{50}$ of 225µM, unlike monomethyl fumarate (MMF). To further clarify the mechanism of action and determine which residue is involved, the same experiment was performed with cysteine mutant forms supposed to be involved in the inhibitory action of DMF. Only one mutation, C599V, displayed the same inhibition as WT after 1h of incubation.

What's more, after an extended incubation time, for $RSK2^{CTKD}$ carrying C436V, C439V, C560V, and C579V mutations, DMF displayed a better potency. This demonstrates that the other cysteines can also be involved in DMF action.

The different results collected in this study enabled the establishment of a strong model for DMF inhibition on the C-terminal kinase domain of RSK2 through the allosteric binding site C599. This new target opens up a new area for the development of specific compounds based on the DMF mechanism of inhibition.

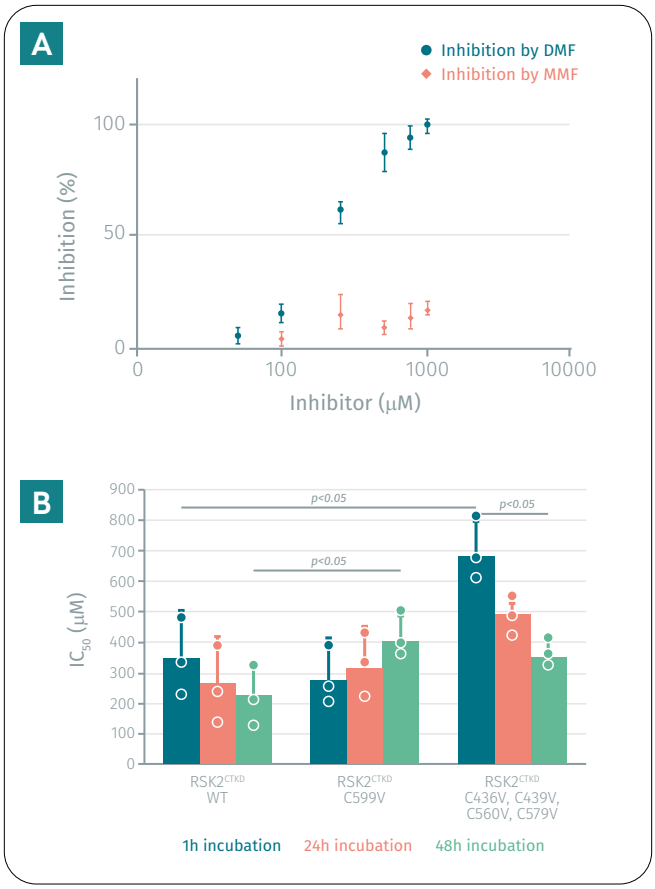



Figure 4: *in vitro* characterization of DMF inhibition on RSK2.

*A. Inhibition of RSK2$^{CTKD}$ by DMF and MMF. Error bars indicate the standard deviation of mean values (n=3, all data points included).*

*B. Time course of DMF inhibition. In vitro determination of apparent $IC_{50}$ values of DMF on RSK2$^{CTKD}$ activity following incubation with DMF, at different time points prior to ERK2 activation (n=3, all data points indicated by circles)(Andersen et al., 2018)*

## Conclusion

Despite their role in numerous diseases, only 80 kinases have been targeted for treatment development. HTRF KinEASE assays have been successfully used over the last decade, and have proved to be highly relevant for the multiple requirements and formats of drug discovery. The publications featured in this note report the effective implementation of such assays for inhibitor selection and mode-of-action assessment applied to various therapeutic areas.

## Bibliography

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