New cell-based HTRF® assays for the exploration of Wnt signaling pathway

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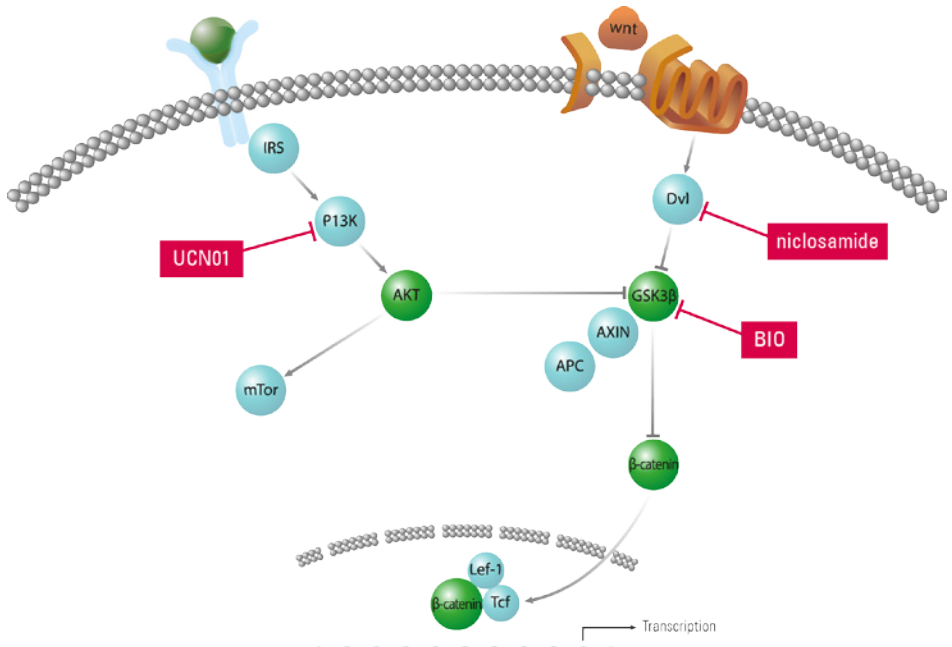
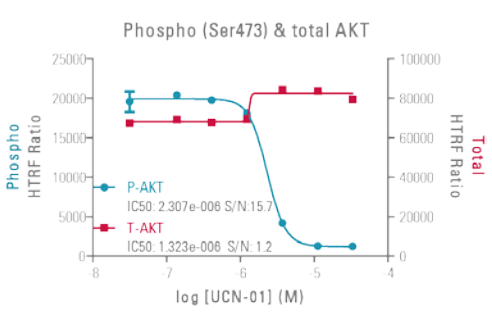
PATHOPHYSIOLOGICAL BACKGROUND - The hallmark of the canonical Wnt pathway is the accumulation and the translocation of β-catenin into the nucleus. In the absence of Wnt signaling, cytoplasmic β-catenin is degraded by a destruction complex, which includes Axin, APC, PP2A, GSK3 and CK1 α. Within this complex, phosphorylation of β-catenin by GSK3 induces its ubiquitination followed by degradation. Conversely, binding of Wnt to its frizzled receptor leads to the disruption of the destruction complex, inducing the translocation of β-catenin into the nucleus where it activates gene transcription. In diseases like colorectal cancers and hepatocellular carcinomas, mutations in the Wnt pathway are frequently observed. More recently a link between Wnt signaling and type II diabetes has also been reported. GSK3, however, not only plays a role in Wnt signaling, but is also involved in a wide range of cellular processes, ranging from glycogen metabolism to cell cycle regulation and proliferation. Hence its inactivation has pleiotropic pathophysiological effects (ref 1).

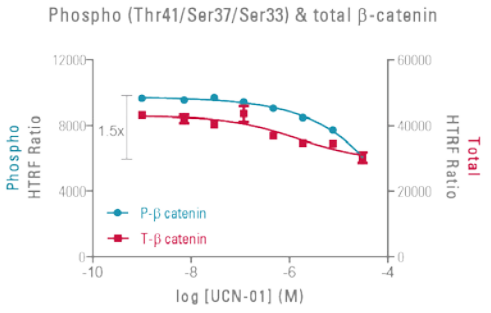
DRUG DISCOVERY AND EXPERIMENTS - Extensive research in stabilizing or destabilizing β-catenin has enabled the discovery of inhibitors (eg IWP2 or XAV939) or activators (e.g. BIO or SB216763) of the Wnt signaling pathway. Moreover recent evidence has indicate a crosstalk between PI3K/AKT/GSK3 and Wnt/β-catenin pathways (refs 2, 3).

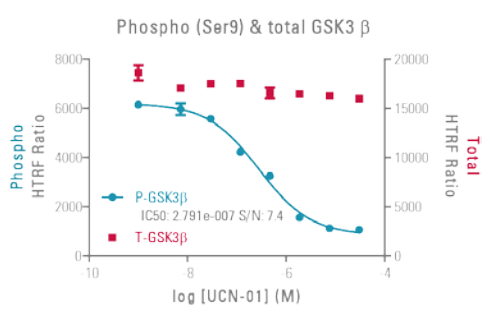
Here we investigate the endogenous AKT/GSK3 and Wnt pathways by analyzing the phospho-readouts upon compound treatment. HEK293 were either treated with a compound, UCN01, targeting the PI3K/AKT/GSK3 pathway, or by niclosamide and BIO which both act on the Wnt pathway. The protein level and phosphorylation of AKT, GSK3, and β-catenin were monitored with the novel HTRF® cell-based phospho-assays.



UCN-01 EFFECT

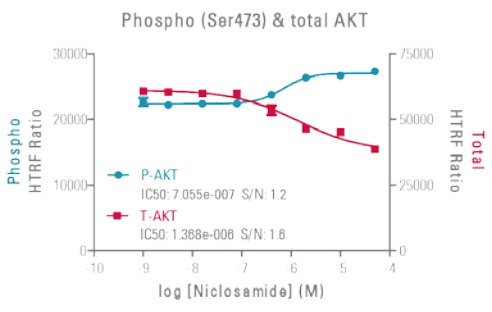


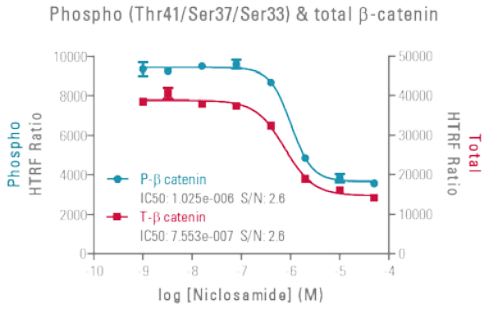


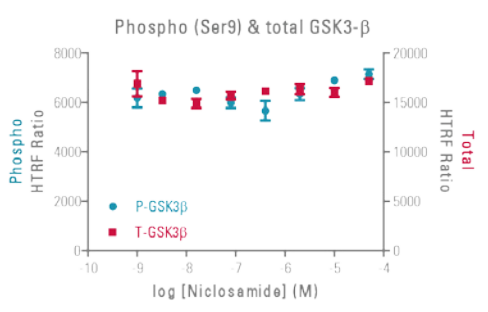


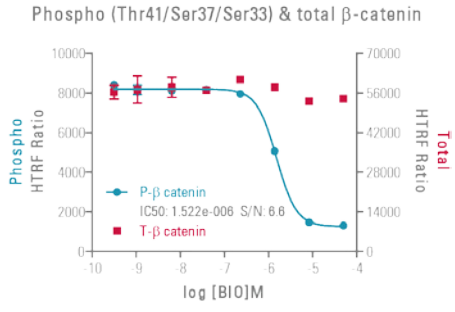
COMMENTS : The inactivation of AKT by UCN01 leads to a marked diminution of GSK3 β phosphorylation (ref 4) and a slight β-catenin downregulation

NICLOSAMIDE AND BIO EFFECTS









COMMENTS : As expected, Niclosamide and BIO downregulate Wnt signaling by a decrease in the β-catenin protein level (ref 5).

EXPERIMENTAL CONDITIONS

100K HEK293 cells were treated with UCN-01 for 6 hours, then stimulated with Insulin 1 µM-30min.

MG-132 was used to prevent β-catenin degradation (5 µM/2h). Results were obtained using the 2 step protocol of the HTRF® cell-based phospho-assays.

RESULTS

Disruption of PI3K/AKT/GSK3 signaling induced a slight decrease in the β-catenin protein level, whereas niclosamide and BIO lead to a significant diminution of Wnt signaling. In addition, we observed an increase in AKT phosphorylation after niclosamide exposure together with a diminution of the total AKT protein level, reinforcing the hypothesis of a crosstalk between the PI3K/AKT and Wnt pathways.

Simplified dissection of the molecular mechanisms of this signaling network, facilitated by the new HTRF® GSK3 and β-catenin cell-based phospho-assays, provides valuable clues for deeper biological understanding and subsequently the development of new therapeutics.

CONCLUSION The exploration of signaling pathways has become essential in many biological areas, from basic to applied research, whether biological target-oriented or focused on disease and the associated pathophysiological state.

Despite numerous technologies supporting pathway investigations, result accuracy combined with rapid and simple assay procedures for all throughputs are still rare.

Now, with more than 40 different HTRF® phospho-assays, Cisbio Bioassays has become the preferred partner when labs seek to improve research productivity, efficacy and reliability in the outcome.

Cisbio's GSK3 and β-catenin cell-based phospho-/total protein assays facilitate screening and characterization of drugs targeting the Wnt-b catenin signaling pathway, offering simplicity and time and cost savings.

The pharmacological validation given here with the reference compounds UCN01 and Niclosamide shows that these assays have the sensitivity and the accuracy required for the detection and robust quantification of readouts, as well as enabling the reliable determination of pharmacological parameters as IC50 values.

Thanks to the simple and rapid « add-and-read » protocol, these tools represent a real improvement over more conventional, time-consuming heterogeneous methods for the development of new drugs.

Moreover, the assays can be easily combined with the broad portfolio of Cisbio cellular phospho-/total protein assays, like phospho-ERK and phospho-AKT, for compound profiling or signaling cross-talk investigation.

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