

## Impedance-based cellular analysis – Z-factor

# Assay validation and Z-factor calculation of GPCR activity with the **Bionas Discovery™ adcon reader**

### INTRODUCTION

The quality and suitability of an assay is of major importance when testing the effectiveness and sensitivity of drugs during drug target identification. The Z-factor calculation is a useful mathematical tool to assess the quality and the effect size of an assay and is therefore used for assay validation and optimization. Here we performed a study with the **Bionas Discovery™ adcon reader** and determined a dynamic Z-factor calculation.

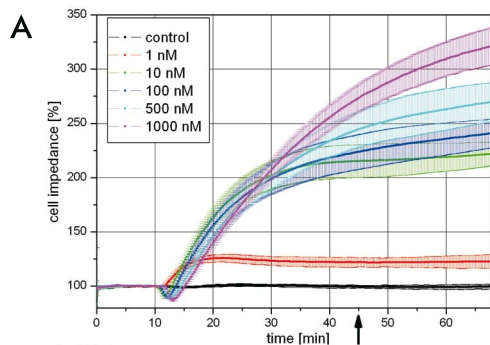
For that purpose, HeLa and U2OS cells were used to investigate the activation of S1P receptors. S1P receptors are G-protein coupled receptors (GPCRs) and are activated by sphingosine-1-phosphate (S1P). S1P is a bioactive sphingolipid metabolite formed by the phosphorylation of sphingosine which is ubiquitously expressed and implicated in the regulation of a variety of physiological cellular processes. Thus, S1P is an eligible measure and positive control for assay performance and Z-factor calculation.

### RESULTS

For assay quality and stability assessment, dynamic Z-factor calculation was performed for HeLa cells (Figure 1) and U2OS cells (Figure 2) with the adcon software. The application of different concentrations of S1P resulted in a dose-dependent increase of the impedance in both cell lines. The dynamic Z-factor calculation provided essential information about the (time-dependent)

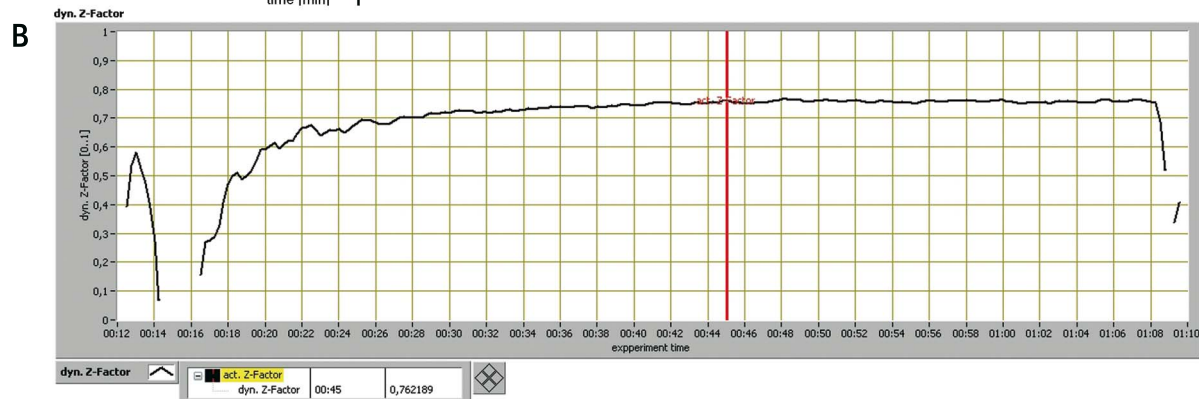
quality of the assay. In general, Z-factor values between 0.5 and 1 define an excellent assay<sup>1</sup>. Within a few minutes, the values reached a stable level above 0.5 which was kept until the end of the experiment.

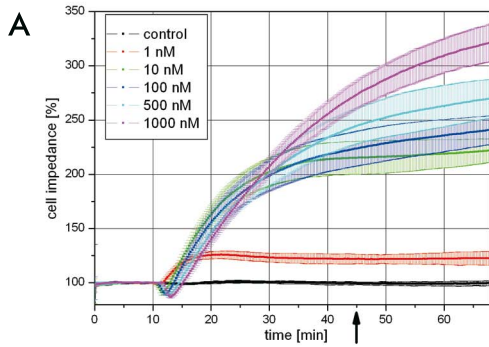
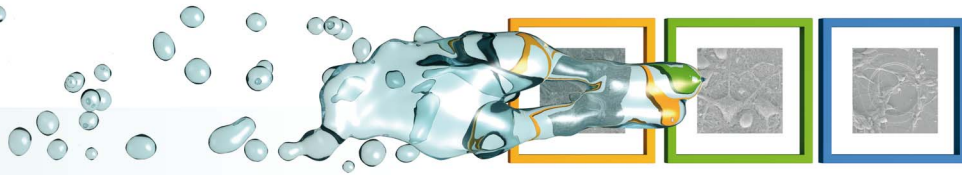
Cell line	Z-factor	Time-point
HeLa	0.76	45 min
U2OS	0.77	8 min



**Fig. 1: HeLa cell assay evaluation.**

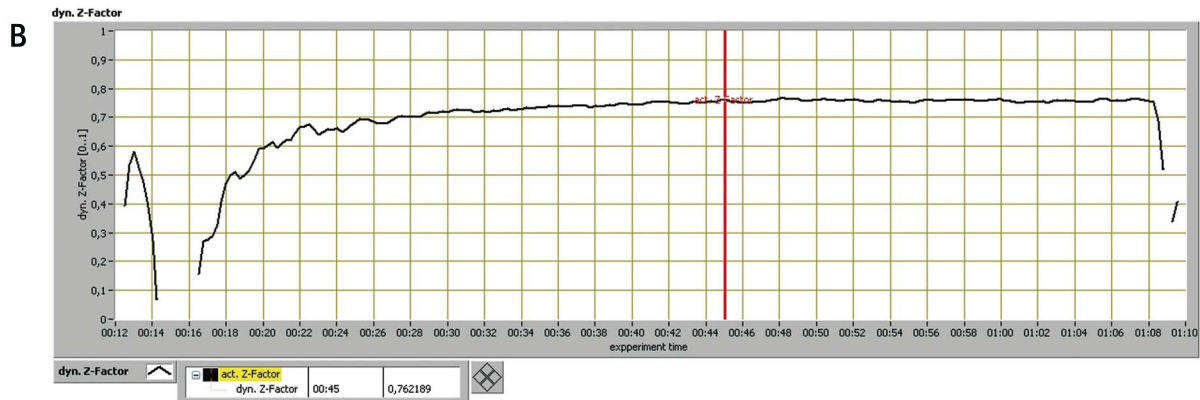
A: Monitoring of the concentration-dependent S1P impedance. The arrow points to the time of best Z-factor determination. B: Dynamic Z-factor calculation for assay quality determination. The red bar indicates the timepoint with the highest Z-factor of 0.76, which was 45 min after adding S1P.





**Figure 2: U2OS cell assay evaluation.**

A: Monitoring of the concentration-dependent S1P impedance. The arrow points to the time of best Z-factor determination. B: Dynamic Z-factor calculation for assay quality determination. The red bar indicates the timepoint with the highest Z-factor of 0.77, which was 7 min after adding S1P.



The major advantage of a real-time dynamic Z-factor presentation is to find the best time for assay analysis, optimizing the length of experiments.

## MATERIALS & METHODS

**S1P assay.** HeLa cells (human carcinoma cell line) and U2OS cells (human osteosarcoma cell line) were seeded in an adcon plate at a density of  $1.5 \times 10^4$  cells/well. Prior to S1P addition, the cultivation medium was changed to Hank's buffer + 0.1% BSA + 20 mM HEPES + P/S. S1P (1 nM – 1000 nM) was added in Hank's buffer during the ongoing measurement and impedance was monitored for the following 60 min.

**Analysis in the Bionas Discovery™ adcon reader.** The Bionas Discovery™ adcon reader consists of the plate station, the analyzer and a PC as user interface. The plate station accommodates the adcon plate in 96-well format with interdigitated electrodes at the bottom of each well for impedance measurement providing information about cell viability, morphology, adhesion/ confluence, proliferation and membrane integrity. Cellular adhesive alterations, e.g. upon stimulation of receptors, are recorded in real-time. Dynamic Z-factor, which is calculated from the sample means and the

sample standard deviation was analyzed with the adcon reader software using a S1P concentration of 1000 nM.

## CONCLUSION

The Bionas Discovery™ adcon reader is a powerful tool to perform cell-based assays of highest quality as shown by Z-factor values higher than 0.7. The real-time monitoring from minutes to hours provides the possibility of a dynamic presentation of the Z-factor. Hence, the best time point for an assay analysis can be chosen to obtain the most sensitive results. Especially in the field of GPCR drug development the knowledge about assay quality and reproducibility is of highest importance.

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**LITERATURE.** 1Zhang JH, Chung DY and Oldenburg KR (1999): A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. J Biomol Screen. 4: 67-73.

