Biomarker Analysis of Gadd34 Induction by Proteasome Inhibiton from Cell Culture & Tumor Lysates Using Acoustic Membrane and Microparticle (AMMP™) technology on the ViBE™ Bioanalyzer from BioScale

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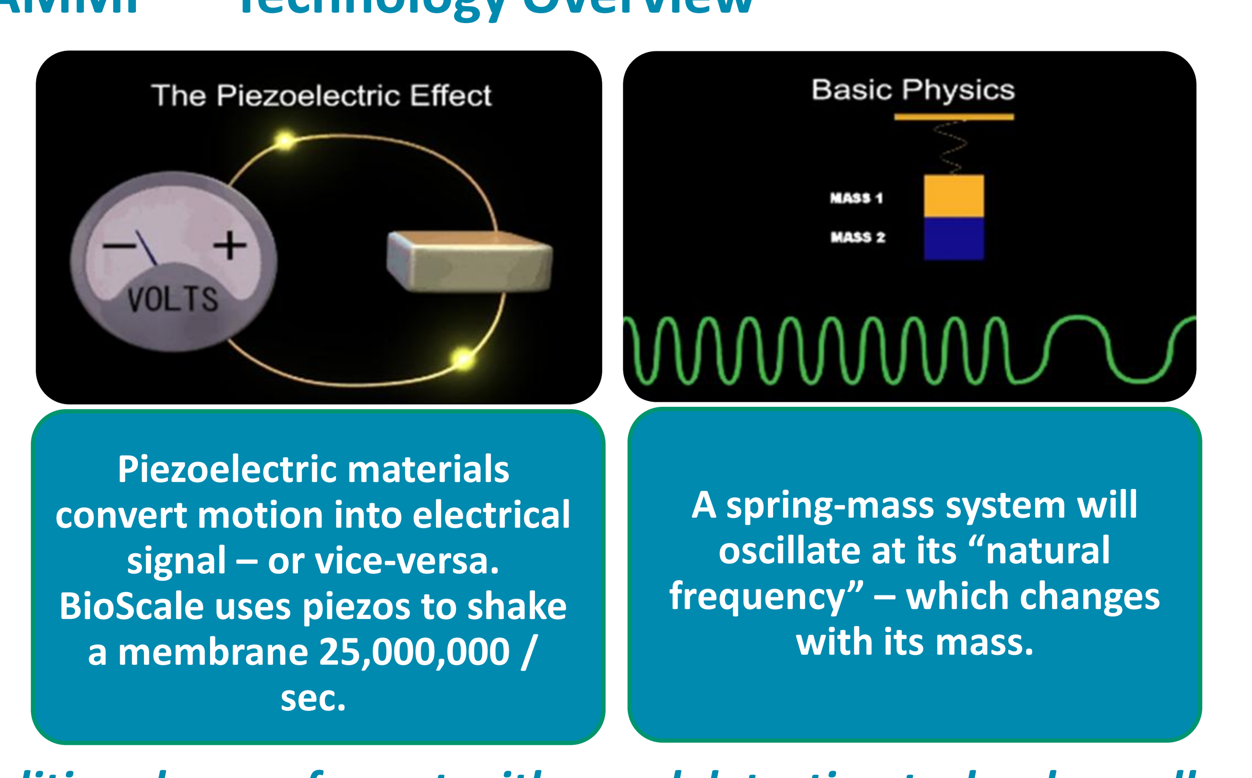
Abstract: Gadd34 or growth arrest and DNA damage protein 34 is a stress-induced protein implicated in the control of protein synthesis and apoptosis(1). Under normal non-stressed conditions Gadd34 is a rapidly degraded protein through the ubiquitin-proteasome pathway(2). Because proteasome inhibition by bortezomib or its analogs induces Gadd34 accumulation we sought to develop a strategy for monitoring increases in protein levels using a novel microfluidic frequency modulating technology from BioScale called AMMP™ detection. Toward that goal, we developed a complementary pair of rabbit monoclonal antibodies against recombinant Gadd34. These antibodies were then affinity tagged and used to generate a standard curve with recombinant Gadd34. Cell culture derived HCT116 or CWR22 xenograft tumor lysates either treated or untreated with proteasome inhibitors were analyzed on the ViBE™ Bioanalyzer and compared to results obtained with either LiCor® Western Blot or AlphaScreen™. Our results show clearly that the BioScale technology compares favorably with data obtained from LiCor® Western Blot and AlphaScreen™. Moreover, the use of the ViBE™ Bioanalyzer eliminates the labor intensive effort of Western Blot analysis and, furthermore, is devoid of the optical and chemical interferences derived from lysates of xenograft tumors observed with AlphaScreen™.

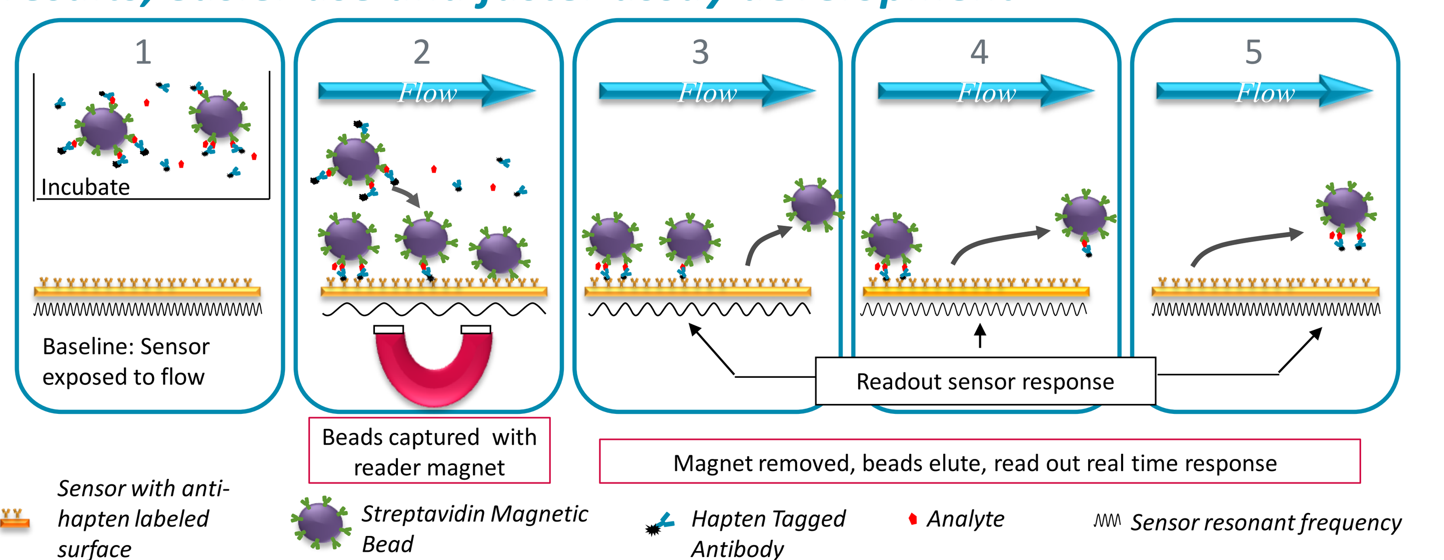
References:

1. **Mammalian GADD34, an apoptosis- and DNA damage-inducible gene**. Hollander MC, Zhan Q, Bae I, Fornace AJ Jr., *J Biol Chem.* 1997 May 23;272(21):13731-7.
2. **Control of cellular GADD34 levels by the 26S proteasome**. Brush MH, Shenolikar S., *Mol Cell Biol.* 2008 Dec;28(23):6989-7000.
3. **Inhibition of eIF2alpha dephosphorylation maximizes bortezomib efficiency and eliminates quiescent multiple myeloma cells surviving proteasome inhibitor therapy**. Schewe DM, Aguirre-Ghiso JA. *Cancer Res.* 2009 Feb 15;69(4):1545-52.

AMMP™ Technology Overview

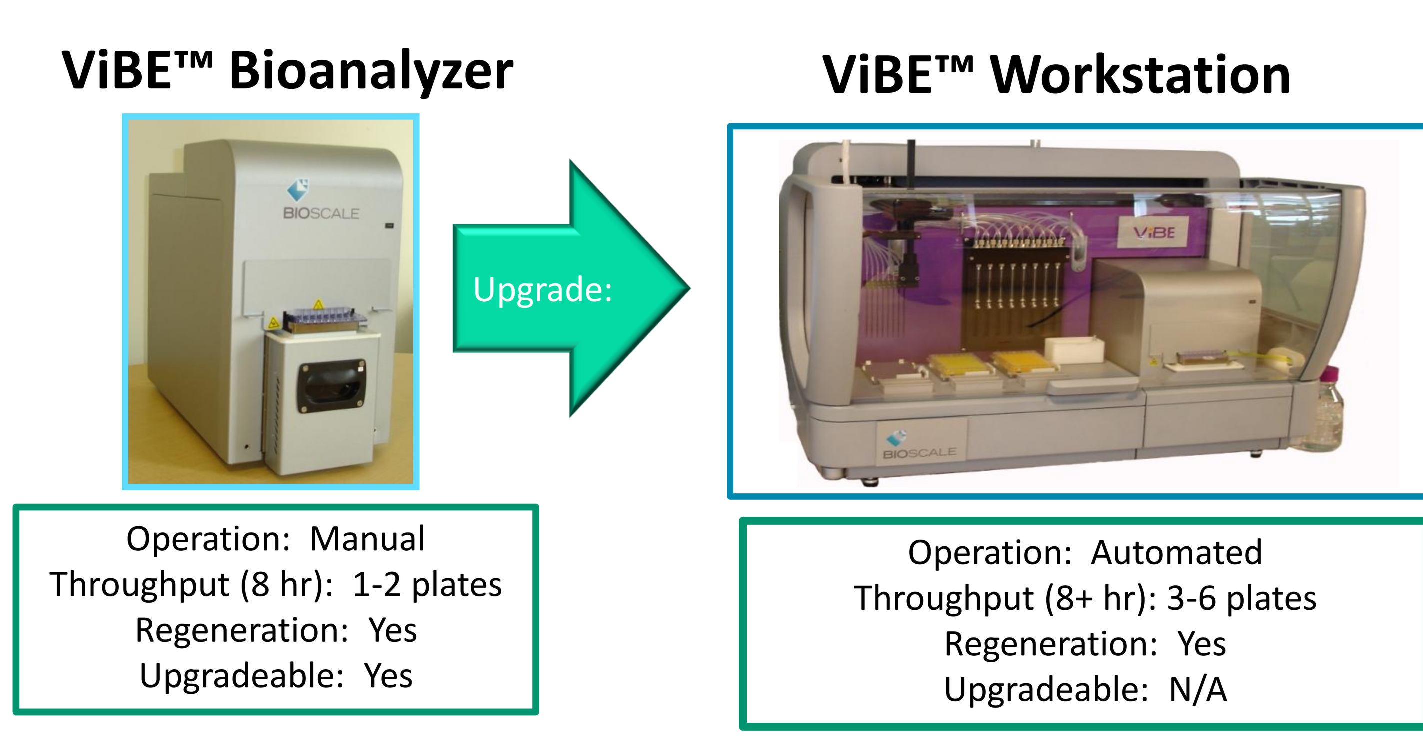


Traditional assay format with novel detection technology allows better results, easier use and faster assay development



1. **Sample is first incubated with streptavidin magnetic capture beads and complementary antibodies each tagged with either a hapten or biotin.**
2. **Beads are collected on the biosensor surface by the applying a magnetic field.**
3. **Beads are briefly washed with buffer while still engaged by the magnet to ensure capture by the anti-hapten antibodies at the membrane surface.**
4. **Magnetic field is released and beads without capture analyte are washed away.**
5. **Under continuing flow, beads elute according to their analyte load.**

Instrumentation:



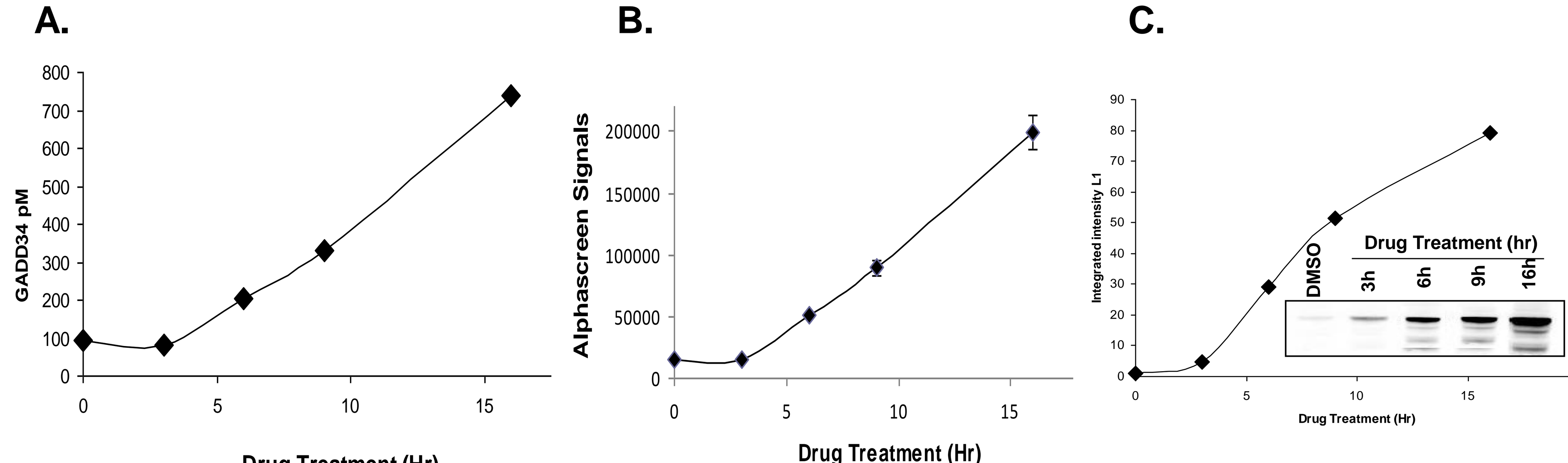


Fig 3. Induction of Gadd34 of HCT116 derived cell culture lysates in the presence or absence of boronate proteasome inhibitor as analyzed by either ViBE™ BioAnalyzer (Panel A) or AlphaScreen™(Panel B) or Western blot LiCor® (Panel C). HCT116 cells were cultured in the presence or absence of 3 nM of a bortezomib analog and lysed in mPER buffer at the times indicated. Protein concentrations were determined by BCA and normalized for sample addition for each assay. The same complementary pairs of anti-Gadd34 antibodies as described in Fig. 1 for the solution based assays(panels A & B) or using anti-Gadd34 antibody(ProteinTech) for the LiCor® assay(panel C) were used. In the case of the ViBE™ assay the AMMP signal was normalized to the rGST-Gadd34 signal from Fig.1 using their commercial software package. Results shown are representative of at least duplicate experiments with standard errors of less than 10%.

Results:

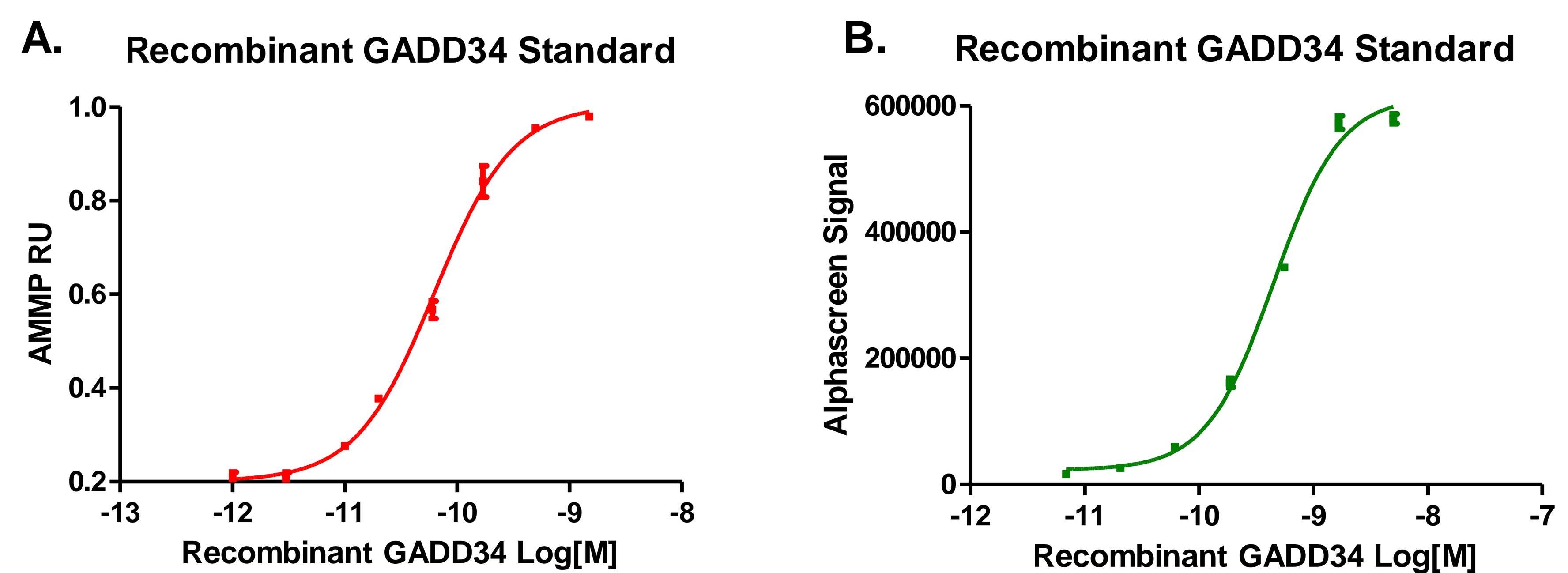


Fig 1. Standard curve of signal obtained by either ViBE™ BioAnalyzer (Panel A) or AlphaScreen™ technology (Panel B) using recombinant GST-Gadd34. Recombinant GST-tagged Gadd34 was titrated over an eight point three-fold dilution series in buffers recommended by the manufacturers. Complementary pairs of anti-Gadd34 antibodies were labeled with either biotin or fluorescein for use with the ViBE™ Bioanalyzer as directed by the manufacturer. For the AlphaScreen assay antibodies were bead captured and used at 10 μg/ml for the donor and acceptors and analyzed on a BMG PheraStar™.

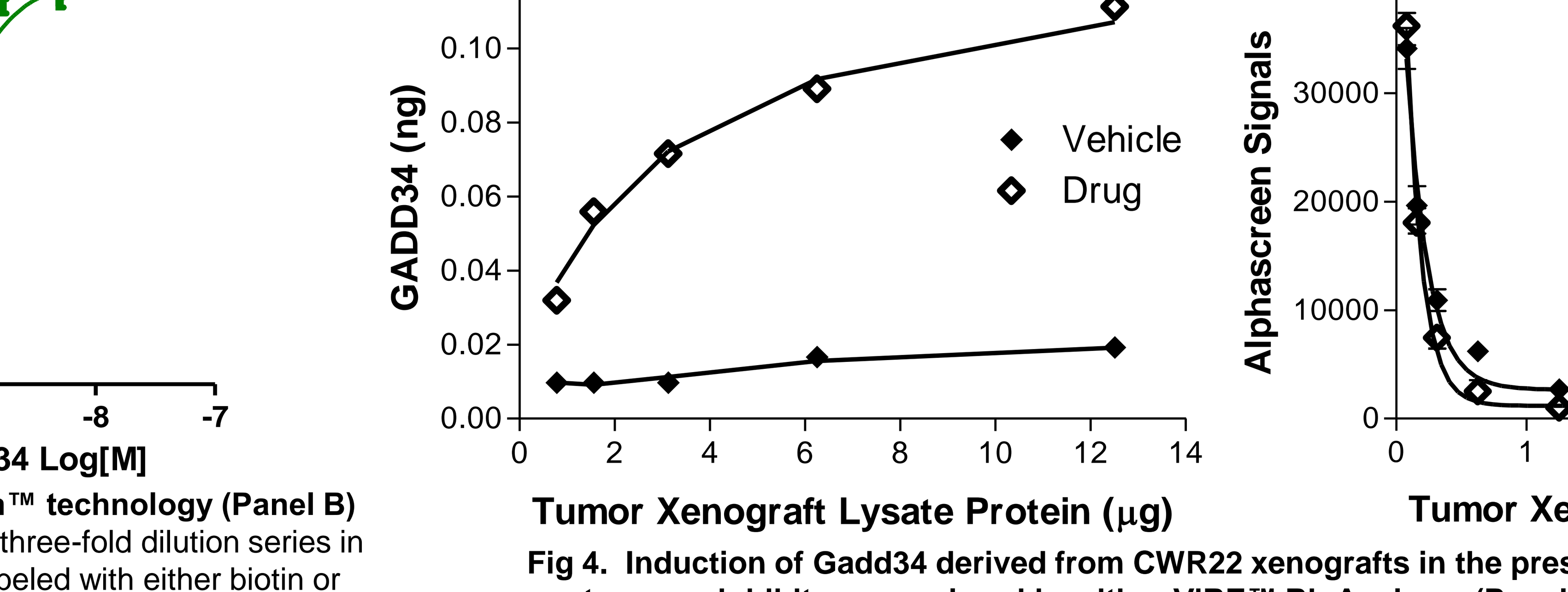


Fig 4. Induction of Gadd34 derived from CWR22 xenografts in the presence or absence of a boronate proteasome inhibitor as analyzed by either ViBE™ BioAnalyzer (Panel A) or AlphaScreen™(Panel B). Tumor bearing mice were treated iv with a bortezomib analog and tumor xenograft samples excised and homogenized following a 16 hr dose. Protein concentrations were determined by BCA. In the case of the ViBE™ assay, the AMMP™ signal was normalized to the rGST-GADD34 signal from Fig.1 using their commercial software package. Note the quenching of the AlphaScreen signal with increasing tumor μg of protein added to the assay. Results shown are representative of at least duplicate experiments with standard errors of less than 10%.

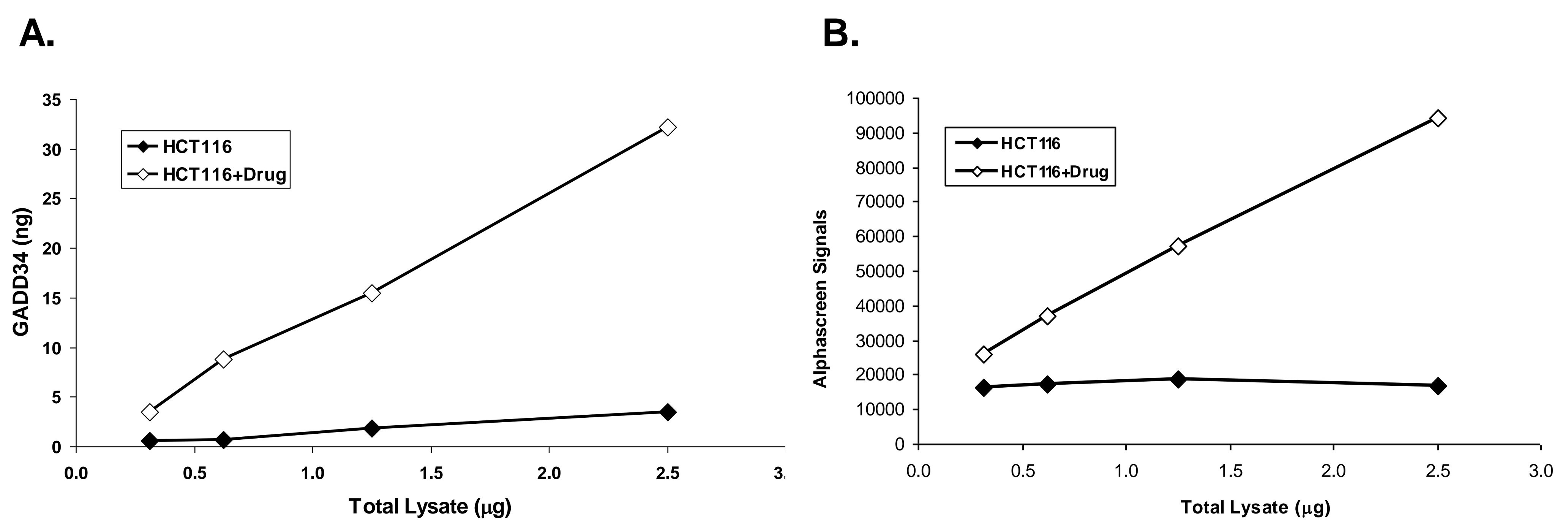


Fig 2. Titration of HCT116 cell culture lysate as analyzed by either ViBE™ BioAnalyzer (Panel A) or AlphaScreen™(Panel B). HCT116 cells were cultured in the presence or absence of 3 nM bortezomib for 16 hrs prior to lysis in mPER buffer(Pierce). Protein concentrations were determined by BCA and titrated using the same complementary pairs of anti-Gadd34 antibodies as described in Fig. 1. In the case of the ViBE™ assay the AMMP signal was normalized to the rGST-Gadd34 signal obtained from Fig.1 using the instrument's software package. Results shown are representative of duplicate experiments with standard errors of less than 10%.

Conclusions:

1. Samples derived from a variety of sources can be readily analyzed using the ViBE™ Bioanalyzer producing the same quality results from recombinant, tissue culture or *in vivo* tumor xenografts.
2. Unlike other modalities, i.e., Western blot or AlphaScreen, the ViBE™ Bioanalyzer affords rapid, reproducible and quantitative analysis of all sample types.