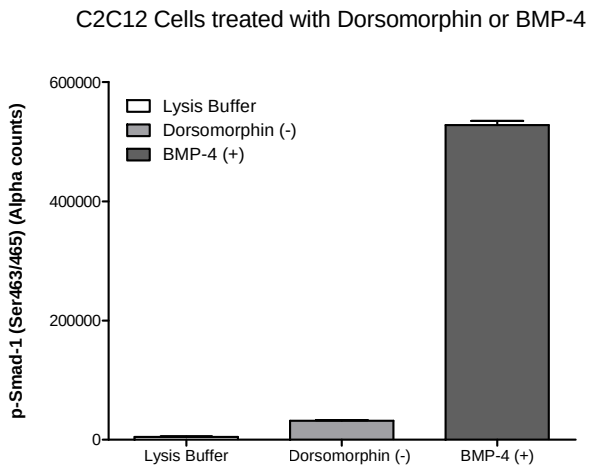
AlphaScreen® *SureFire*® phospho-Smad1 (Ser463/465) Kit

Catalog #
TGRSM1S500
TGRSM1S10K
TGRSM1S50K

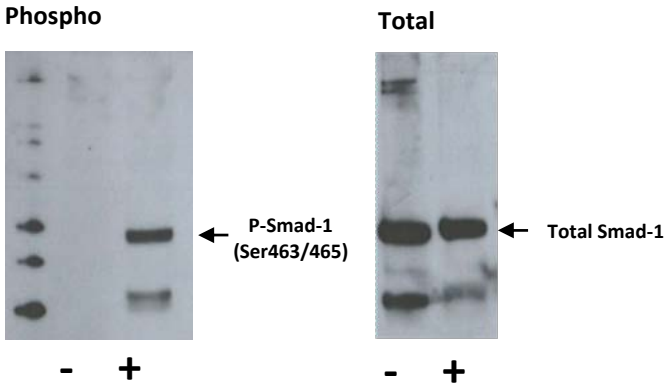
Bone morphogenetic protein (BMPs) receptors belong to the TGFbeta-receptor superfamily, and bind BMPs to regulate a range of cellular processes, via the Smad signaling pathway. BMP binding to the receptors induces multimerization and activation of the receptors, which can then phosphorylate Smad1 at Ser463 and Ser465, and Smad5 and Smad8 at corresponding C-terminal sites. Activated Smads dimerize with Smad4, translocate to the nucleus, and stimulate transcription of particular genes.

Specificity:

A



B



Note:
The Phospho antibody also detects Smad 5 p-Ser463/465 and Smad 8 p-Ser426/428.

Based on sequence homology the total antibody may also detect Smad 5 and Smad 8.

Cell Handling Protocol:

2 x T175 Flasks of C2C12 Cells were seeded at 50K cells/mL (56mL/flask) on Day 0. On Day 3 the cells were serum starved for 90 minutes and then treated with **50µM Dorsomorphin (-)** for 60 minutes or **50ng/mL BMP-4 (+)** for 30 minutes at 37°C.

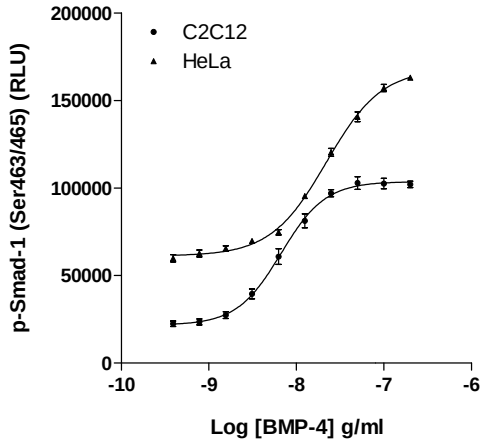
Assay Protocol:

The media was then removed from the flasks and the cells were lysed in 12mL/flask of 1x Lysis Buffer with agitation. Lysates were spun to remove cellular debris, aliquotted and frozen at -20°C.

Thawed lysates were then analysed for p-Smad-1 (Ser463/465) and / or Total Smad-1 using either the standard 2-step AlphaScreen® *SureFire*® protocol at room temperature (4µL lysate / 384-Proxiplate™ well) **(A)** or by Western Blot (11.25µL lysate / well) using the same antibodies used for the *SureFire*® Reaction Buffer **(B)**.

A

BMP-4-mediated Smad-1 (Ser463/465) in C2C12 and HeLa Cells



Agonist Dose Response Curves:

Cell Handling Protocol:

C2C12 Cells were seeded at 50K/mL and Hela Cells were seeded at 100K/mL in a 96 well plate (200µl/well giving 10K and 20K cells/well respectively). Cells were grown overnight at 37°C.

Assay Protocol:

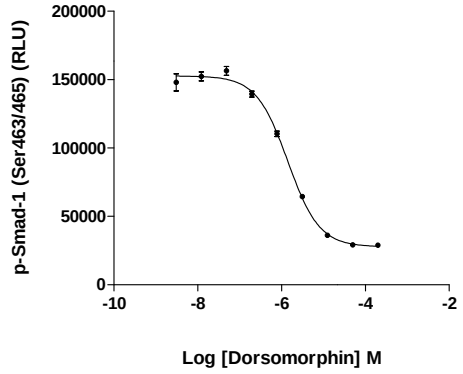
The following day the media was removed and the cells were serum starved for 60 minutes at 37°C and then dose stimulated with various concentrations of **BMP-4 (A)** for 30 minutes at 37°C.

The media was removed from the wells and the cells were lysed with 50µL/well 1X Lysis Buffer with shaking for 10 minutes.

Lysates (4µL) were then transferred to a 384-well Proxiplate™ and analysed for phospho-Smad-1 (Ser463/465) using the standard 2-step AlphaScreen® *SureFire*® Protocol at room temperature.

B

Inhibition of BMP-4-mediated p-Smad-1 (Ser463/465) in C2C12 Cells



Inhibitor Dose Response Curve:

Cell Handling Protocol:

C2C12 Cells were seeded at 100K/mL in a 96 well plate (200µl/well giving 20K cells/well). Cells were grown overnight at 37°C.

Assay Protocol:

The following day the media was removed and the cells were dose inhibited with various concentrations of **Dorsomorphin (B)** for 30 minutes at 37°C then stimulated with **BMP-4** (50ng/ml) for 30 minutes at 37°C.

The media was removed from the wells and the cells were lysed with 50µL/well 1X Lysis Buffer with shaking for 10 minutes.

Lysates (4µL) were then transferred to a 384-well Proxiplate™ and analysed for phospho-Smad-1 (Ser463/465) using the standard 2-step AlphaScreen® *SureFire*® Protocol at ~22°C.