



Standard Test Method for *In Vitro* Biological Activity of Recombinant Human Bone Morphogenetic Protein-2 (rhBMP-2) Using the W-20 Mouse Stromal Cell Line¹

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1. Scope

1.1 This test method describes the method used and the calculation of results for the determination of the *in-vitro* biological activity of rhBMP-2 using the mouse stromal cell line W-20 clone 17 (W-20-17). This clone was derived from bone marrow stromal cells of the W++ mouse strain.²

1.2 This test method (assay) has been qualified and validated based upon the International Committee on Harmonization assay validation guidelines³ (with the exception of inter-laboratory precision) for the assessment of the biological activity of rhBMP-2. The relevance of this *in vitro* test method to *in vivo* bone formation has also been studied. The measured response in the W-20 bioassay, alkaline phosphatase induction, has been correlated with the ectopic bone-forming capacity of rhBMP-2 in the *in vivo* Use Test (UT). rhBMP-2 that was partially or fully inactivated by targeted peracetic acid oxidation of the two methionines was used as a tool to compare the activities. Oxidation of rhBMP-2 with peracetic acid was shown to be specifically targeted to the methionines by peptide mapping and mass spectrometry. These methionines reside in a hydrophobic receptor binding pocket on rhBMP-2. Oxidized samples were compared alongside an incubation control and a native control. The 62, 87, 98, and 100 % oxidized samples had W-20 activity levels of 62, 20, 7, and 5 %, respectively. The incubation and native control samples maintained 100 % activity. Samples were evaluated in the UT and showed a similar effect of inactivation on bone-forming activity. The samples with 62 % and 20 % activity in the W-20 assay demonstrated reduced levels of bone formation, similar in level with the

reduction in W-20 specific activity, relative to the incubation control. Little or no ectopic bone was formed in the 7 and 5 % active rhBMP-2 implants.

1.3 Thus, modifications to the rhBMP-2 molecule in the receptor binding site decrease the activity in both the W-20 and UT assays. These data suggest that a single receptor binding domain on rhBMP-2 is responsible for both *in-vitro* and *in-vivo* activity and that the W-20 bioassay is a relevant predictor of the bone-forming activity of rhBMP-2.

1.4 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Terminology

2.1 *rhBMP*—recombinant human bone morphogenetic protein.

2.2 *GDF*—growth and differentiation factor.

3. Summary of Test Method

3.1 In this test method, the mouse stromal cell line W-20-17 is used as a target cell line for rhBMP-2. The W-20-17 cells exhibit increased alkaline phosphatase activity in response to rhBMP-2. Optical density at 405 nm of the p-nitrophenol generated from the alkaline phosphatase substrate is used as a measure of alkaline phosphatase enzyme level. The test method is performed in a 96-well plate format. A similar test method based upon the same cell line has been developed using chemiluminescent detection of alkaline phosphatase.⁴

¹ This test method is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.42 on Biomaterials and Biomolecules for TEMPs.

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² Thies, R. S., Bauduy, M., Ashton, B. A., Kurtzberg, L., Wozney, J.M., and Rosen, V., "Recombinant Human Bone Morphogenetic Protein-2 Induces Osteoblastic Differentiation in W-20-17 Stromal Cells," *Endocrinology*, Vol 130, 1992, pp. 1318–1324.

³ Guideline for Industry, ICH-Q2A Text on Validation of Analytical Procedures, November 1996, International Committee on Harmonization, March 1995, <http://www.fda.gov/cder/guidance/index/htm>.

⁴ Blum, R. S., Li, R. H., Mikos, A.G., and Barry, M.A., "An Optimized Method for the Chemiluminescent Detection of Alkaline Phosphatase Levels During Osteodifferentiation by Bone Morphogenetic Protein 2," *Jour. Cellular Biochem*, Vol 80, 2001, pp. 532–537.