OTHER

QUANTITATIVE RECOVERY OF BMP-2 AFTER GRINDING OF DEMINERALIZED BONE WITH PRECELLYS® EVOLUTION

Affinergy, LLC, Morrisville, NC, USA

/ CONTEXT

Bone Morphogenetic Protein 2 (BMP-2) is one of the most potent growth factors involved in bone growth and repair. Since it is present at low levels, its extraction and quantitation from bone is challenging. To evaluate the quantitative recovery of recombinant BMP-2 after a stringent grinding cycle in a Precellys® Evolution, we spiked commercial, demineralized cancellous bone with the protein, pulverized the samples, and measured the level of BMP-2 in the ground sample with an industry standard immunoassay.

/ MATERIALS

- **Bead beating Homogenizer:** Precellys® Evolution (Cat #: 02520-300-RD000) with Cryolys® cooling unit (Cat #: 05068.200.RD001)
- **Lysing Kits:** Hard tissue homogenizing CK28R_2mL (Cat #: KT03961-1-007.2)
- **Tissue Samples:** Human cancellous bone
- **Extraction Buffer:** 4 M Guanidine Hydrochloride and 50 mM EDTA in Tris/HCl buffer pH 7.6 containing *Complete* protease inhibitor
- Recombinant BMP-2 from Medtronic INFUSE kit (Medtronic Cat #: 7510600)
- Quantikine BMP-2 Immunoassay Kit (R&D Systems Cat #: DBP200)

/ RESULTS

The extract from the unspiked demineralized cancellous bone did not yield any signal in the Quantikine assay, indicating that its BMP-2 content was below the detection level of the assay.

For the extract of the spiked bone sample, the Quantikine assay measured a level of BMP-2 of 227 ng/ml, which corresponded to a **101% recovery of the BMP-2 spiked into the sample.**

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/ PROTOCOL

Samples: 50mg of bone was spiked with 225ng of BMP-2. Unspiked material was processed alongside the spiked one as a baseline control.

Homogenization: 1mL of extraction buffer was added to each 2mL Precellys® tubes. Samples were ground by running 5 cycles of 20 sec at 5,000 rpm, with 30 sec break between cycles.

Cryolys® cooling unit: The temperature of the cooling unit was set to 4 °C during the grinding process.

Grind samples were rotated at 8 rpm on a rotisserie shaker for 18h before being centrifuged at 10,000 rcf for 2 min. The supernatants were analyzed for BMP-2 content with a Quantikine assay.

/ CONCLUSION

Stringent grinding of demineralized cancellous bone in guanidine hydrochloride extraction buffer using a Precellys® Evolution instrument allowed to obtain the quantitation of BMP-2 by a standard immunoassay. Therefore grinding of bone with a Precellys® Evolution instrument is a viable approach to pulverize the material for extraction of BMP-2 prior to immunoassay. The Cryolys cooling unit ensured low temperature during the process which allowed to prevent protein denaturing.

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エムエス機器株式会社
口東京 〒162-0805 東京都新宿区矢来町 113 番地 TEL（03）3235-0661（代）/ FAX（03）3235-0669
口大阪 〒532-0005 大阪市淀川区三国本町2丁目12番4号 TEL（06）6396-0501（代）/ FAX（06）6396-0508

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