

MATRIX OI®

IN-VIVO OSTEOINDUCTION ASSAY IN ATHYMIC RAT

Every donor lot (Tables A1-A5 indicate up to 3 lots) is tested in 2 different rats (# indicates rats: 19, 20, and 21) which are euthanized on Day 28. Histological criteria for evidence of osteoinduction include the presence of: Chondrocytes, Osteoblasts, Cartilage, Bone Marrow, and New Bone (see last column). X verifies presence. Implants displaying a Grade score of #1 or greater are considered osteoinductive.

TABLE A1: PRELIMINARY ANIMAL DATA					
Animal Number	Test Article Lot #		Initial Weight	Terminal Weight	Gained Weight
	Left Side	Right Side			
19	CRT 130009-DCM	CRT 130014-DCM	198.5	290.1	91.6
20	CRT 130009-DCM	CRT 130022-DCM	240.1	306.7	66.6
21	CRT 130014-DCM	CRT 130022-DCM	232.6	293.8	61.2

TABLE A2: MACROSCOPIC OBSERVATIONS						
Animal Number	Left Side			Right Side		
	Location	Size	Shape	Location	Size	Shape
19	F	A	ND	F	A	ND
20	F	A	ND	F	A	ND
21	F	A	ND	F	A	ND

KEY:
F: Found in correct location
A: Average
ND: No defined shape

TABLE A3: SUMMARY OF PATHOLOGY REPORT - LOT # : CRT130009-DCM								
Animal Number	Size	Chondroblasts /cytes	Osteoblasts /cytes	Cartilage /osteoid	New Bone	Bone Marrow	Original DBM	Grade (0-4)
19	LL	X	X	X	X	X	X	1
20	LL	X	X	X	X	X	X	1

TABLE A4: SUMMARY OF PATHOLOGY REPORT - LOT # : CRT130014-DCM								
Animal Number	Size	Chondroblasts /cytes	Osteoblasts /cytes	Cartilage /osteoid	New Bone	Bone Marrow	Original DBM	Grade (0-4)
21	LL	X	X	X	X	X	X	2
19	RL	X	X	X	X	X	X	1

TABLE A5: SUMMARY OF PATHOLOGY REPORT - LOT # : CRT130022-DCM								
Animal Number	Size	Chondroblasts /cytes	Osteoblasts /cytes	Cartilage /osteoid	New Bone	Bone Marrow	Original DBM	Grade (0-4)
20	RL	X	X	X	X	X	X	1
21	RL	X	X	X	X	X	X	2

KEY:
X: Presence of elements
-: Element not present
LL: Left leg
RR: Right leg

OSTEOINDUCTIVE IN-VIVO LOT VERIFIED

every lot . . . every time

MATRIX OI®

IN-VITRO ALKALINE PHOSPHATE INDUCTION ASSAY

Table 1

ACCESSION NUMBER 13-003794, 13-003796, 13-003797 AND 13-003798					
MATRIX OI®					
	Sample Number	Concentration Tested (mg/well)	Protein Dilution Factor	AP Dilution Factor	Specific Activity AP Units/mg Protein
N/A	BMP Control	N/A	N/A	N/A	15.433
	Cell Lysate Control	N/A	N/A	N/A	<LOQ
13-003794 CRT130023-DCM-OI-2	0001	20	10x	Neat	151.257
13-003796 CRT130032-DCM-OI-2	0001	20	10x	5	206.684
13-003797 CRT130035-DCM-OI-2	0001	20	10x	5	219.062
13-003798 CRT130041-DCM-OI-2	0001	20	10x	5	283.482

Matrix OI® (Table 1) test articles 13-003794, 13-003796, 13-003797, and 13-003798 indicate the Specific Activity AP Units/mg Protein values exceed the BMP control by a factor of up to 19 times greater.

TEST SYSTEM DESCRIPTION

CellRight's Matrix OI® has demonstrated the ability to induce ectopic new bone formation in the soft tissue of experimental animals. Some of this activity can be attributed to the presence of stimulatory proteins, including bone morphogenic proteins (BMPs). BMPs irreversibly induce differentiation of perivascular mesenchymal-type cells into osteoprogenitor cells. BMPs can also act in-vitro to activate a differentiation pathway in the pluripotent myoblast C2C12 cell line. C2C12 cells stimulated by these compounds produce increased levels of alkaline phosphatase. This assay was designed to quantitatively detect the presence of these stimulatory compounds in bone products by their ability to induce alkaline phosphatase activity in C2C12 cell culture.