



## Work Package Number: WP4

### Deliverable Number: D4.3

# Agreed protocols for culture conditions of in vitro and ex vivo models used by different partners and method of analysis

<i>Phenotyping of Cellular Models</i>	
Unit:	RSD: CSD:

<i>OsteoClast Primary Culture (human, mouse, other)</i>				
Culture Conditions	Phenotype		Function	Other
	TRAcP histochemistry	mRNA of OCs markers	Bone resorption assay	
		TRAcP Cathepsin K RANK Calcitonin Receptor	Pit assay CTX Osteologic discs	

<i>OsteoBlast Primary Culture (human, mouse, other)</i>				
Culture conditions	Phenotype		Function	Other
	ALP histochemistry	mRNA of OBs markers	Mineralization assay	
		ALP Runx2 Osterix Osteocalcin	vonKossa staining Alizarin red Calcium content	



<i>Multipotent/pluripotent cells (human, mouse, other)</i>				
<i>Differentiation to osteoblasts</i>				
<b>Culture conditions</b>	<b>Phenotype</b>		<b>Function</b>	<b>Other</b>
	N. of colonies	ALP histochemistry	Mineralization assay	
			vonKossa staining Alizarin red Calcium content	
<i>Differentiation to chondrocytes</i>				
<b>Culture conditions</b>	<b>Phenotype</b>			
	N. of colonies	Type II collagen	Alcian blue staining	Other
<i>Differentiation to adipocytes</i>				
<b>Culture conditions</b>	<b>Phenotype</b>			
	N. of colonies	Oil red histochemistry	Other	

<i>Osteocyte Primary Culture (human, mouse, other)</i>				
<b>Culture conditions</b>	<b>Phenotype</b>			<b>Other</b>
	Stellate morphology	mRNA of osteocyte markers	IHC of osteocyte markers	
		DMP1 Sclerostin ALP negative	DMP1 Sclerostin ALP negative	



<i>Chondrocyte Primary Culture (human, mouse, other)</i>				
<b>Culture conditions</b>	<b>Phenotype</b>			<b>Other</b>
	Alcian Blue staining	mRNA of chondrocyte markers	IHC of chondrocyte markers	
		COL2A1 COL11A1 SOX9 Aggrecan	COL2A1 COL11A1 SOX9 Aggrecan	

<i>iPS (human, mouse, other)</i>				
<b>Culture conditions</b>	<b>Phenotype</b>			<b>Other</b>
	mRNA of iPS genes	In vitro growth	Multipotent Commitment	In vivo teratoma induction
	Oct3/4 KLF4 cMyc Sox2	ES-like colonies	mRNA expression of lineage-specific genes	Histological analysis of ectodermal, mesodermal and ectodermal tissues