## BENCH-TOP PROTOCOLS for eCube Tissue RNA Mini Kit

eCube Tissue RNA Mini Kit * for tissue		* for tissue	eCube Tissue RNA Mini Kit		* for cultured cell
<ul> <li>* Things to do before starting</li> <li>1. Pipet a required volume of XPRB Buffer to another RNase-free container and add 10ul β-mercaptoethanol (β-ME) per 1ml XPRB Buffer before use.</li> <li>2. For 50preps, add 60ml ethanol (96-100%) and for 200preps, add 250ml ethanol (96-100%) to Wash Buffer 2. For 300preps, add 200ml ethanol (96-100%) to each Wash Buffer 2 when first open.</li> <li>3. All centrifuge steps are done at full speed (14,000 rpm or 10,000 x g) in a microcentrifuge.</li> </ul>		<ul> <li>* Things to do before starting</li> <li>1. Pipet a required volume of XPRB Buffer to another RNase-free container and add 10ul β-mercaptoethanol (β-ME) per 1ml XPRB Buffer before use.</li> <li>2. For 50preps, add 60ml ethanol (96-100%) and for 200preps, add 250ml ethanol (96-100%) to Wash Buffer 2. For 300preps, add 200ml ethanol (96-100%) to each Wash Buffer 2 when first open.</li> <li>3. All centrifuge steps are done at full speed (14,000 rpm or 10,000 x g) in a microcentrifuge.</li> </ul>			
Grind the Sample (up to 30mg)		Cells grown suspension/mo	n in nolayer		
	<ul> <li>1. Add 350ul of XPRB Buffer (ß-ME added) to the sample and shear by passing lysate through a 20-G needle syringe 10 times. Incubate at room temperature for 5 min.</li> <li>2. Transfer the sample mixture to Filter Column.</li> <li>3. Centrifuge for 2 min.</li> <li>4. Transfer the supernatant from Collection Tube to a new micro-centrifuge tube (not provided).</li> <li>5. Add 1 volume of 70% ethanol to the clear lysate and mix well by vortexing.</li> <li>6. Transfer the sample (including any precipitate) to XPRB Mini Column.</li> </ul>			<ol> <li>Harvest 1-5 x 10<sup>6</sup> cells by centrifuge at 300 x g for 5 min</li> <li>Add 350ul of XPRB Buffer (B-ME added) to the cell pellet and vortex vigorously to lyse the cells. Incubate at room temperature for 3 min.</li> </ol>	
			2. Transfer the sample mixture to <b>Filter Column</b> .		
			<ul> <li>3. Centrifuge for 2 min.</li> <li>4. Transfer the supernatant from Collection Tube to a new micro-centrifuge tube (not provided).</li> <li>5. Add 1 volume of 70% ethanol to the clear lysate and mix well by vortexing.</li> <li>6. Transfer the sample (including any precipitate) to XPRB Mini Column.</li> </ul>		
	8. Add <b>500ul of Wash Buffer 1</b> to XPRB Column.	Centrifuge for 1 min then discard the flow-through.		8. Add <b>500ul of Wash Buffer 1</b> to XPRB Column.	7. Centrifuge for 1 min then discard the flow-through.
	9 10. Add <b>750ul of Wash Buffer 2</b> to XPRB Column.	<ol> <li>Centrifuge for 1 min then discard the flow-through.</li> </ol>		10. Add <b>750ul of Wash Buffer 2</b> to XPRB Column.	<ul> <li>9. Centrifuge for 1 min then discard the flow-through.</li> </ul>
	11	. Centrifuge for <b>1 min</b> then discard the flow-through.			11. Centrifuge for <b>1 min</b> then discard the flow-through.
_< >-	12. Ce	ntrifuge for <b>3 min</b> to dry column	_<>>		12. Centrifuge for <b>3 min</b> to dry column
	<ol> <li>Add 50ul of RNase-free Water to the membrane center of XPRB Mini Column.</li> <li>Stand the column for 1 min.</li> </ol>			<ol> <li>Add 50ul of RNase-free Water to the membrane ce Stand the column for 1 min.</li> </ol>	enter of XPRB Mini Column.
_< >-		14. Centrifuge for 2 min	-<>>-		14. Centrifuge for <b>2 min</b>
Ť	15. You can get pure RNA.			15. You can get pure RNA.	

Please consult the eCube Tissue RNA Mini Kit Handbook before using these protocols for the first time.



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