

**WERF EPHect Standard Operating Procedures:**

**Collection, processing, and storage of ectopic and eutopic endometrium, myometrium, and peritoneum tissue samples**

PROTOCOL STEPS	All sample types							
Sample collection meta-data (incl. excision method)	See Becker et al (Fertil Steril 2014;102:1213-22) and complete biospecimen questionnaire							
Preparation	Prepare and label vials/tubes for transfer and storage of samples as much as possible							
Specimen transfer to the laboratory	As soon as possible; keep on ice until preservation/storage; If transport likely to take >15 mins, consider immediate snap-freezing or RNA stabilising procedures in/near theatre.							
<i>SD=Standard</i> <i>RM=Required minimum</i>	<b>SD</b>				<b>RM</b>			
Sample processing	<ul style="list-style-type: none"> <li>Record time of starting sample processing</li> <li>Rinse tissue with PBS (except for RNA studies)</li> <li>Weigh tissue samples; if tissues to be stored in separate pieces, prioritize: 1<sup>st</sup> snap freezing, 2<sup>nd</sup> RNA stabilizing solution [see detailed protocol for examples of commercially available products], 3<sup>rd</sup> universal molecular fixative or formalin fixation.</li> </ul>				<ul style="list-style-type: none"> <li>Record time of starting sample processing</li> <li>Rinse tissue with PBS (except for RNA studies)</li> <li>Weigh tissue samples; if tissues to be stored in separate pieces, prioritize: 1<sup>st</sup> snap freezing, 2<sup>nd</sup> RNA stabilizing solution [see detailed protocol for examples of commercially available products], 3<sup>rd</sup> universal molecular fixative or formalin fixation.</li> </ul>			
	Type of processing							
	Fresh tissue		Snap-frozen tissue		RNA stabilising + freezing		Fixed tissue	
<i>SD=Standard</i> <i>RM=Required minimum</i>	SD	RM	SD	RM	SD	RM	SD	RM
	<ul style="list-style-type: none"> <li>Store in fluid media/PBS at 4°C (max 24 hrs);</li> <li>Transfer to waterbath (37°C) for cell culture</li> </ul>	<ul style="list-style-type: none"> <li>Store in fluid media/PBS at 4°C (max 24 hrs)</li> </ul>	<ul style="list-style-type: none"> <li>Store in vial with screw top gasket;</li> <li>Snap freeze in liquid nitrogen (LN<sub>2</sub>) within 15 mins;</li> <li>Record time.</li> </ul>	<ul style="list-style-type: none"> <li>Store in vial with screw top gasket;</li> <li>Snap freeze in LN<sub>2</sub> as soon as possible;</li> <li>Record time.</li> </ul>	<ul style="list-style-type: none"> <li>Within 15 mins of collection, cut samples to ≤0.5cm + immerse in RNA stabilising solution<sup>1</sup> in screw top gasket vial.</li> <li>Record time;</li> <li>Store at 4°C for 24 hours prior to freezing.</li> </ul>	<ul style="list-style-type: none"> <li>As soon as possible, cut samples to ≤0.5cm + immerse in RNA stabilising solution<sup>1</sup> in screw top gasket vial.</li> <li>Record time;</li> <li>Store at 4°C for 24 hours prior to freezing.</li> </ul>	<ul style="list-style-type: none"> <li>Within 15 mins of collection, mount flat in Histokinette™ cassette in 20ml 10% NBF<sup>2</sup>;</li> <li>Record time; Transport at room temperature or place on ice.</li> <li>Leave in NBF for max 24 hours;</li> <li>Transfer to graded alcohol solutions + paraffin embedding.</li> </ul>	<ul style="list-style-type: none"> <li>Within 1 hr of collection, mount flat in Histokinette™ cassette in 20ml 10% NBF<sup>2</sup>;</li> <li>Record time. Transport at room temperature or place on ice;</li> <li>Leave in NBF for max 24 hours;</li> <li>Transfer to graded alcohol solutions + paraffin embedding.</li> </ul>

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<i>SD=Standard RM=Required minimum</i>	<b>SD</b>	<b>RM</b>
<b>Storage</b>	<ul style="list-style-type: none"> <li>• Store in LN<sub>2</sub> freezer.</li> </ul>	<ul style="list-style-type: none"> <li>• Store in -80°C or lower freezers.</li> </ul>
<b>Freezer check</b>	<ul style="list-style-type: none"> <li>• Alarm system setup on all freezers.</li> <li>• Biweekly human check.</li> </ul>	<ul style="list-style-type: none"> <li>• Biweekly human check.</li> </ul>
<b>Data recording Checklist</b>	<ul style="list-style-type: none"> <li>• Record protocol, specifying which steps are adhered to (standard or minimum).</li> <li>• For each sample, record:               <ul style="list-style-type: none"> <li>▪ Date and time of collection (Date: __/__/__ and __: __am/pm).</li> <li>▪ Start time of sample processing in the laboratory (Date: __/__/__ and __: __am/pm).</li> <li>▪ Type and number of lesions prepared.</li> <li>▪ Date and time sample is stored into freezer (Date: __/__/__ and __: __am/pm).</li> <li>▪ Any variations or deviations from the SOP, problems, or issues.</li> <li>▪ Any freeze-thaw event that occurs with a sample for any reason.</li> </ul> </li> <li>• Keep a log of bi-weekly freezer checks.</li> </ul>	

<sup>1</sup> Follow and record manufacturer’s protocol.

<sup>2</sup> NBF= neutral buffered formalin.