Technical Note

# CREATING *A-FLX™* FFPE CELL PELLET BLOCKS

## INTRODUCTION

This document provides a guide for using the A-FLX™ services to create **Formalin Fixed Paraffin Embedded (FFPE)** cell pellet blocks.

## PRODUCT FEATURES

### CONSISTENCY AND HOMOGENEITY

The A-FLX™ FFPE pellets are the world's most consistent FFPE cell pellets. Every step of creating a cell pellet is strictly controlled, from cell culture, fixation, embedding, FFPE processing, and sectioning. Using the A-FLX™ process, cells are homogeneously distributed across the entire FFPE block, as a result, FFPE sections cut from different blocks and different batches will have highly consistent cell number and density and perform consistently when used in different applications.

### FLEXIBILITY

The A-FLX™ FFPE cell pellet process is extremely flexible and allows customers to produce cell pellets that best fit their applications.

- Flexible cell density: the standard
- Flexible cell pellet size:
- Flexible fixation

## SAMPLE REQUIREMENT

### STARTING FROM FOR PRODUCING FFPE TISSUE OR CELL PELLET BLOCKS

FFPE is the most commonly used specimen type in clinical practices. A tissue or cellular sample is fixed with formalin (typically 10% NBF), processed using a fully automated tissue processor, and embedded into paraffin block. Cellular morphology and most bio-molecules can be well preserved in FFPE blocks for extensive periods of time.

Sending samples for FFPE embedding:

1. For tissue specimens:
    1) After fixing for 24 hours in **10% NBF**, briefly wash the samples with **1XPBS** (minimum of 20:1 solution/sample volume ratio) two times.
    2) Briefly wash with **70% ethanol** (minimum of 20:1 solution/sample volume)
    3) Add fresh **70% ethanol** (minimum of 20:1 solution/sample volume ratio)
    4) The samples can be shipped cold (with ice packs) in **70% ethanol** via overnight shipping.

2. For producing *A-FLX™* FFPE cell pellet using cultured cells, PBMCs, or cells from other bodily fluids:
    1) After harvesting, remove culture medium or fluid by centrifugation at 250 RCF for 5min in a conical tube.
    2) Wash cells one time using **1XPBS** by centrifugation at 250 RCF for 5min.

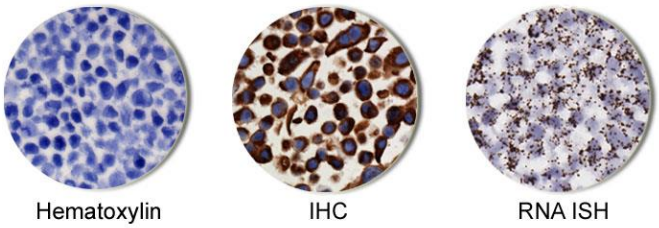
3) Remove **1XPBS** and re-suspend cells thoroughly in **10% NBF** by pipetting, be sure to break large cell clumps.
   ***Note:*** *Small clumps may occasionally form depending on cell type, culturing condition, trypsinization condition, etc. It is normal, and even extensive pipetting won't completely break small cell clumps.*
4) Fix cells for 24hr at ambient temperature or in a temperature controlled incubator (24-27°C) with rotation.
5) To ship fixed cells, use one of the two methods below:
   Method 1 – Shipping in 1XPBS:
   a. Pellet cells by centrifugation at 250 RCF for 5min. Remove fixative and then re-suspend in **1XPBS** (approximately 20:1 solution/pellet ratio) thoroughly by pipetting or vortexing.
      ***IMPORTANT:*** *Do not perform additional washes. The 1XPBS solution will contain residual fixative, which keeps sample stable during shipping while not leading to over-fixation.*
   b. Ship samples cold (with ice packs) in **1XPBS** via overnight shipping.

   Method 2 – Shipping in 70% ethanol:
   a. Pellet cells by centrifugation at 250 RCF for 5min. Remove fixative and then re-suspend in **70% ethanol** (approximately 20:1 solution/pellet ratio) thoroughly by pipetting or vortexing.
   b. Ship samples cold (with ice packs) in **70% ethanol** via overnight shipping.

   ***Note:*** *Shipping fixed cells in 1XPBS or 70% ethanol using the methods described above does not typically lead to significantly different assay performance when the samples are made into FFPE blocks.*

3. About *A-FLX*™ FFPE cell pellets:
   *A-FLX*™ is a propriety technology developed by Acepix Biosciences that produces FFPE cell pellets with:

- High homology
- High cross-block and lot-to-lot consistency
- Excellent shape regularity
- Customizable cell density and pellet size




## SECTION 2 – FOR PRODUCING CYTOSPIN SLIDES

Cytospin protocol is commonly used in clinical and research settings for preparing mono cell layers onto microscopic slides from:

- PBMC
- Bodily fluids, such as saliva and urine
- Cytology samples

- Cultured cells

Sending samples for cytospin:

1) Sample fixation:

   a. Fix cells in appropriate fixatives according to lab protocols. Typical fixatives include **10% NBF, 50-100% methanol,** and **70% ethanol.**
   b. Pellet cells by centrifugation at 250 RCF for 5min. Remove fixative and then re-suspend in **70% ethanol** (approximately 20:1 solution/pellet ratio) thoroughly by pipetting or vortexing.
   c. Ship samples cold (with ice packs) in **70% ethanol** via overnight shipping.

   ***Note:*** *Most alcohol-based fixatives will keep samples stable for at least 48hr if stored cold. Avoid shipping samples in progressive cross-linking agents, such as formaldehyde, as fluctuation in temperature and duration of shipping may cause variation in fixing strength.*

## ABOUT ACEPIX BIOSCIENCES

Acepix Biosciences, Inc. is an innovator in developing and providing high quality biological specimens for research and diagnostics. The company also provides high quality, fast turnaround, and economical services in the field of histo-pathology, biomarker discovery, and translational research. Acepix Biosciences is headquartered in San Jose, California. For more information, please visit www.acepixbio.com.

## SHIPPING

Please ship all packages to:
ATTN: Receiving Department
Acepix Biosciences, Inc.
2355 Paragon Drive, Suite C
San Jose, CA, 95131