

# Blood Plasma Collection and Storage

## Materials:

- 1) Blood Collection tubes. **Cat. # 366450**, from Becton Dickinson ([www.bd.com](http://www.bd.com))



(13 x 100 mm x 7.0 ml BD Vacutainer™ glass whole blood tube. Lavender conventional closure. Paper label. Additive: K<sub>3</sub>EDTA 15% solution, 0.081 ml, 12.15 mg (100/bx, 1000/ca))

- 2) BHT; Butylated hydroxytoluene **Sigma Cat. # B1378**; 100g bottle ([www.sigma.com](http://www.sigma.com))

FW = 220.4;

Stock = 20 mM in ethanol; 1mM = 220.4 mg/L; 20 mM = 4.408 g/L; or

20 mM = 0.04408 g/10 mL ethanol; Aliquot 5 μL/mL = 100 μM (fc);

Stable @ 4 °C in ethanol

Note: fc (final concentration)

- 3) Protease Inhibitor Cocktail for use with mammalian cell and tissue extracts DMSO solution. **Sigma Cat. # P8340** ([www.sigma.com](http://www.sigma.com)).
- 4) Scienceware\* Round Bubble racks. ([Microcentrifuge Tube Racks](http://Microcentrifuge Tube Racks)). Available from Fisher Scientific ([www.fishersci.com](http://www.fishersci.com)) **Cat. No 14-792-14** (Bel-Art No.: F18875 0400). Specifications: No of places 8, Disc Diameter 66 mm.



For use in beakers or ice buckets. Hold 1.5mL tubes. Discs are 1/4 in. thick (0.6cm). Circular hole pattern with either single or double ring depending on size. Legs, 3/4 in. long (1.9cm). Made from polymethylpentene

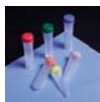
- 5) **Nalgene\* Polyethylene Dewar Flasks**. Available from Fisher Scientific ([www.fishersci.com](http://www.fishersci.com)) **Cat. No 10-194-100A** (Nalgene No.: 4150 1000). Specifications: Capacity 1 L, height 23 cm, I.D at mouth 9.5 cm.



Break- and chemical-resistant, withstand temperatures from -196° to +100°C (-321° to +212°F). High-density polyethylene Dewar flasks with HDPE covers are well suited for cryogenic work. Can be used for dry ice solvent and hot baths. Handy for storing frozen biological samples. Double walls. Annulus is filled with CFC-free foamed urethane to minimize heat leakage. All flasks (except 101/2-qt. size) have polyethylene-coated steel handle. Built-in spout, finger grip on bottom, and ribbed sides

for safer handling.

- 6) Nonsterile microcentrifuge tubes. Available from Fisher Scientific ([www.fishersci.com](http://www.fishersci.com)) **Cat. No 02-681-343** (Nonsterile Skirted-bottom tubes, 2 mL, Natural color). Screw caps sold separately.



Polypropylene tubes suitable for vapor-phase liquid nitrogen storage, boiling and autoclaving. Durable. Withstand g-forces up to 18,000–30,000xG (RCF); autoclaving to 120°C and freezing to -80°C. External ribs on the tubes help to position and lock tubes into racks and allow single-handed opening and closing operation. All tubes and caps have frosted writing area and are autoclavable. Choose conical or skirted tubes in 0.5, 1.5, or 2.0mL sizes, in natural or amber colors

- 7) Screw caps with O-ring for the microcentrifuge tubes. Available from Fisher Scientific ([www.fishersci.com](http://www.fishersci.com)) **Cat. No 02-681-358** (Natural color)
- 8) Fiberboard Storage boxes. Available from Fisher Scientific ([www.fishersci.com](http://www.fishersci.com)) **Cat. No 11-678-24A** (Height 2", 5.25"L x 5.25"W). Supplied with covers.
- 9) Fiberboard Box dividers (Use with fiberboard storage boxes to divide into grids for vial storage). Available from Fisher Scientific ([www.fishersci.com](http://www.fishersci.com)) **Cat. No 11-678-24C** (Vial size 12 mm, Grid size 10 x 10).
- 10) **Falcon\* BlueMax\* Jr. 15mL Graduated Tubes**. Available from Fisher Scientific ([www.fishersci.com](http://www.fishersci.com)) **Cat. No 14-959-70C** (Falcon No.:352097). Specifications: 15 mL, 17 x 120 mm, polypropylene.



**With conical bottoms.** Tubes are constructed from clear polypropylene or high optical quality polystyrene. Graduations run from 1 to 15mL in 0.5mL increments. Large white writing area allows for easy sample identification. Graduations and writing area resist many commonly used solvents. Come with polyethylene dome-seal screw caps for a positive seal over full circumference. Tubes are sterile, nonpyrogenic and packaged in bags or foam racks made from expanded polystyrene. 14-959-53A comes with one rack.

#### Procedure:

- Collect blood in 7 ml vacutainer™ blood collection tubes (lavender top) containing EDTA (10.5 mg). See attached instructions (page 3) for “[Preparing a Quality Sample](#)” from Becton Dickinson technical bulletin. ASAP, proceed as follows:
- Incubate tubes of blood upright at room temperature for 30 min, allowing cells to settle to the bottom of the tube. A light yellow (preferably) to light red color liquid will appear in the upper layer.
- Transfer the upper layer (*no red blood cell contamination!*) to a Falcon 15 mL tube (Fisher Cat. # 14-959-70C). Add 5 µL/mL of BHT and 10 µL/mL of protease inhibitor cocktail (Sigma, Cat.# P8340). Mix gently 5 - 6 times by inversion as described on page 3. **Do not vortex**
- Remove remaining blood cells by centrifugation at 2500 rpm (1300 x g) for 20-30 min at 4 °C. Aliquot 2 mL of the supernatant into 8 vials (250 µL/vial) (Fisher Scientific **Cat. No 02-681-343**) with screw caps (Fisher Scientific **Cat. No 02-681-358**). Also, when removing the plasma, do not pipette close to the buffy coat to prevent contamination from white blood cells! Label the vials.
- To store the samples, flush the vials with argon gently, seal with screw caps, quench-freeze by placing the 8 vials into a Scienceware\* Round Bubble Rack (Fisher Scientific **Cat. No 14-792-14**) and then immersing the bottom of the rack and the tubes in liquid nitrogen in a **Nalgene\* Polyethylene Dewar Flask** (Fisher Scientific **Cat. No 10-194-100A**) for 1 min and then store them in Fiberboard Storage Boxes (Fisher Scientific **Cat. No 11-678-24A**) with Fiberboard Box Dividers (Fisher Scientific **Cat. No 11-678-24C**) at -80 °C. To hold the rack while immersing in liquid nitrogen you might use a long tweezer or try inserting the central peg of the rack snugly into a narrow i.d. tygon tube cut to the appropriate length.

# BD Vacutainer™ Plastic Blood Collection Tubes

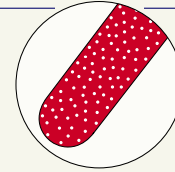
## Preparing a Quality Sample

requires immediate mixing following collection

1

### See the unique additive

All BD Vacutainer plastic tubes have either anticoagulant or clot activator within the interior of the Vacutainer tube.



2

### Draw correct volume of blood

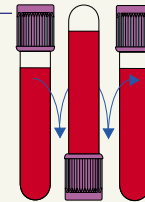
allowing the vacuum in the tube to be exhausted.

3

### Gently invert

All BD Vacutainer plastic tubes require immediate mixing following collection.

Please refer to the chart below for recommendations.



4

### Process as usual

BD Vacutainer™ Tube Type	Closure Color	Number of Inversions
EDTA	Lavender	8-10
*Citrate	Light Blue	3-4
SST with gel	Tiger (Red Gray) or Gold	5
Serum	Red	5
Sodium Fluoride	Gray	8-10
Heparin	Green	8-10

\* Plastic version not yet available for sale or distribution in the U.S.

Handle all biologic samples and blood collection "sharps" (lancets, needles, luer adapters and blood collection sets) according to the policies and procedures of your facility. Obtain appropriate medical attention in the event of any exposure to biologic samples (for example, through a puncture injury) since they may transmit viral hepatitis, HIV (AIDS), or other infectious diseases. Utilize any built-in used needle protector if the blood collection device provides one. BD does not recommend resheathing used needles, but the policies and procedures of your facility may differ and must always be followed. Discard any blood collection "sharps" in biohazard containers approved for their disposal.