# Applicable Laboratory(s)):

[x]  North Carolina Baptist Hospital (NCBH)

[ ]  Lexington Medical Center (LMC)

[ ]  Davie Medical Center (DMC)

[ ]  Wilkes Medical Center (WMC)

[ ]  High Point Medical Center (HPMC)

[ ]  Westchester

[ ]  Clemmons

# Procedure Statement

Monoclonal antibody-based cancer therapies are under development. Some of these therapies may interfere with blood bank tests. Guidelines are developed to determine the best approach to testing in the presence of these new drugs. One of these methods utilizes DTT to cleave CD38 off of the red blood cell membrane.

# Scope

Procedure Owner/Implementer: Blood Bank Management

Procedure Prepared by: Julie Simmons

Who Performs Procedure: Blood Bank Staff

# Definitions

1. Procedure: A process or method for accomplishing a specific task or objective.
2. WFBH Lab System: Wake Forest Baptist Lab System is a health system that includes Wake Forest Baptist Medical Center and all affiliated organizations including Wake Forest University Health Sciences (WFUHS), North Carolina Baptist Hospital (NCBH), Lexington Medical Center (LMC), Davie Medical Center (DMC), Wilkes Medical Center (WMC), High Point Medical Center (HPMC), Lab at Westchester and Lab at Clemmons.

# Sections

1. Preparation of Alkaline Stock Solution, pH 9
2. Preparation of Phosphate-Buffered Saline, pH 8
3. Preparation of 0.2M DTT
4. Preparation of DTT Treated Red Cells

# Procedure

1. **Preparation of Alkaline Stock Solution, pH 9**

Chemical Risk Assessment: low

Biological Risk Assessment: low

Protective Equipment: Lab coat, gloves

Reagents: Na2HPO4 (Sodium phosphate dibiasic), DI water

Supplies: glass beaker/container for mixing

Equipment: scale

Specimen Requirements: NA

| **STEPS** | **INSTRUCTIONS** |
| --- | --- |
| **1.0**  | **Determine whether or not the following stock solution needs to be prepared.**1. Look in reagent refrigerator
2. If found, ensure expiration date is in the future and that there are at least 15mL left in the container. Go to Section II. Preparation of Phosphate-Buffered Saline, pH 8
3. If not found, go to next step
 |
| **2.0** | **Dissolve 2.27g Na2HPO4 (Sodium Phosphate Dibasic) in 100 mL dI water.** Note: This 0.16M solution of dibasic phosphate salt (anhydrous) has pH of 9.0. |
| **3.0** | **Properly label this solution using a reagent label as follows: Alkaline Stock Solution, pH 9.** *Refer to BB-LABELS-0016 Reagent Label*1. Expiration of alkaline stock solution is one year from preparation. Store at 4C.
 |

1. **Preparation of Phosphate-Buffered Saline, pH 8**

Chemical Risk Assessment: low

Biological Risk Assessment: low

Protective Equipment: Lab coat, gloves

Reagents: Alkaline stock solution, PBS

Supplies: glass beaker/container for mixing, pH strips, pipettes

Equipment: NA

Specimen Requirements: NA

| **STEPS** | **INSTRUCTIONS** |
| --- | --- |
| **1.0** | **Add Alkaline Stock Solution, pH 9 (prepared in Section I) one mL at a time to 150 mL of PBS (pH 7) until a pH of 8 has been reached.** Note: This should be approximately 8-10mL |
| **2.0** | **Properly label this solution using a reagent label as follows: Phosphate-Buffered Saline, pH 8.** *Refer to BB-LABELS-0016 Reagent Label*1. Expiration of Phosphate-Buffered Saline, pH 8 is one year from preparation. Store at 4C.
 |

1. **Preparation of 0.2M DTT**

Chemical Risk Assessment: low

Biological Risk Assessment: Low

Protective Equipment: Lab coat, gloves

Supplies: 15mL conical tubes

Reagents: DTT, Phosphate buffered saline pH 8

Equipment: NA

Specimen Type: NA

| **STEPS** | **INSTRUCTIONS** |
| --- | --- |
| **1.0** | **Dissolve 5 grams of DTT powder in 160 mL of Phosphate-Buffered Saline, pH 8 (prepared in Section II)** |
| **2.0** | **Depending on the volume of red cells needed, divide into:**1. **8mL aliquots using 15mL conical tubes (should be approximately 20 aliquots)**

 ***OR***1. **4mL aliquots using 15mL conical tubes (should be approximately 40 aliquots)**

Note:Can also be divided into 1 mL cryovials for selected cell or patient DTT treatment if needed |
| **3.0** | **Properly label the tubes using reagent labels.** *Refer to BB-LABELS-0016 Reagent Label*1. Expiration of prepared DTT is one year from preparation. Store at -18C or colder.
 |
| **4.0** | **Document Lot #, Original expiration date, Date frozen, and # tubes/volume frozen on Frozen Commercial Log in the Stroma/DTT/Frozen RBC binder** *Refer to BB-FORMS-0241 Frozen Commercial Log* |

1. **Preparation of DTT Treated Red Cells**

Chemical Risk Assessment: low

Biological Risk Assessment: low

Protective Equipment: Lab coat, gloves

Reagents: 0.9% PBS at pH of 7.3

DTT (1ml frozen aliquots in Freezer 12)

Donor Red Cells: Red cells positive for K. Red cells positive for E.

Anti-Kell and Anti-E

Supplies: 12x75 mm glass test tubes

Disposable pipettes

Equipment: Light magnifying lamp

Serofuge or CW3 centrifuge

Specimen Requirements: Red cells to be tested

| **STEPS** | **INSTRUCTIONS** | **CHANGE /** **APPROVAL** |
| --- | --- | --- |
| **1.0** | **Label 12x75 test tube for cells being treated with identifying information. Control K positive cell and E positive cell will always be tested.**1. 3% Reagent red cells: Lot number, vial number
2. Donor unit: Unit number or sticker
	1. Determine if 3-cell screen or panel cells or patient cells need to be treated based on testing ordered.
	2. Prepare a 2-5% red cell suspension if using a donor unit.
 |  |
| **2.0** |

|  |  |  |
| --- | --- | --- |
| **Quantity Desired** | **2-5% red cell suspension** | **Comments** |
| 1ml aliquot | 1. Place 1ml of 2-5% red cell suspension in tube.
2. Centrifuge for 60 seconds.
3. Label a tube for each aliquot with cell number and lot number.
4. Remove supernatant and SAVE in appropriately labeled tube
5. This will be used to resuspend the cells once DTT treated.
 |  |
| 2ml aliquot | 1. Place 2 ml of 2-5% red cell suspension in tube.
2. Centrifuge for 60 seconds.
3. Label a tube for each aliquot with cell number and lot number.
4. Remove supernatant and SAVE in appropriately labeled tube.
5. This will be used to resuspend the cells once DTT treated.
 | **Prepared weekly** |
| 1 drop to test | 1. Add 2-3 drops of 2-5% red cells to tube labeled in step 1.
 | **Prepared as** **Needed** |

 |  |
| **3.0** | **Wash red cells one (1) time with PBS saline.**3.1 Fill each tube ¾ full with PBS saline.3.2 Centrifuge for 60 seconds.3.3 Pipet off saline and discard. |  |
| **4.0** | **Add 4 volumes of thawed DTT to volume of red cells selected and MIX.** 4.1 For 3 drops of red cells, use 12 drops of DTT4.2 For 1 ml of red cells, use 4 mls of DTT) 4.3 For 2mls of red cells, use 8 mls of DTT4.4 DTT is stored in 1ml aliquots (approximately 20 drops) in Freezer 12.  |  |
| **5.0** | **Incubate tubes from step 4 at 36-38C for 30 to 45 minutes in waterbath (NOT dry incubator).** |  |
| **6.0** | **Centrifuge at 3400-3600 rpm and remove supernatant after incubation.** |  |
| **7.0** | **Wash four times with PBS saline.** 7.1 Repeat steps 3.1 to 3.3 a total of four (4) times with each tube. 7.2 NOTE: Slight hemolysis may occur.  a. IF hemolysis hue is >5 repeat the process using a small volume of DTT (2-3 volumes instead of 4 volumes) |  |
| **8.0** | **Resuspend the cells to a 2% to 5% suspension in PBS.**

|  |  |
| --- | --- |
| **Volume** | **Steps** |
| 1 ml aliquots | Add the saved supernatant from step 2 back onto the cells to make 2-5% |
| 2 ml aliquots |
| 1 drop aliquot | Add PBS if necessary.  |

 |  |
| **9.0** | **Test DTT-treated control cells with appropriate antibody antisera according to manufacturer’s directions and record on form: Weekly DTT Treated Screening cell QC.**9.1 Test Kell positive treated cell with anti-K.9.2 Test E positive treated cell with anti-E |  |
| **10.0** | **Interpretation**10.1 Continue testing with treated cells if both controls Pass.10.2 Repeat treatment of cells if EITHER control FAILS.

|  |  |  |
| --- | --- | --- |
| **Controls** | **PASS** | **FAIL** |
| K+ DTT treated cell + anti-K | Negative | Positive |
| E+ DTT treated cell + anti-E | Positive | Negative |

 |  |
| **11.0** | **Label the aliquots from step 8 with the following information on separate cryogenic test tubes (or equivalent):**11.1 Cell number and Lot number11.2 Preparation Date11.3 Expiration Date (7 days) |  |
| **12.0** | **Store appropriate cells treated in appropriate refrigerator.**  |  |

# References

Judd’s Methods in Immunohematology

# Related procedures/policies

BB-SOP-0164

# Attachments/Linked documents (title 21)

# Revision Dates: Review Change Summary as represented in Title 21.