Tissue Dissociation Reagent

BD Tumor Dissociation Reagent

A Custom RUO Product

Material number
661563

To order contact:
BDB_Custom_Orders@bd.com

Research applications include:

RESEARCH APPLICATIONS

- Dissociation of solid tumor samples into single cell suspension (optimized for breast cancer)
- Recommended tissue input size for 1 reagent aliquot: 0.05-1.0 grams
- Sample preparation for downstream single cell analyses such as FACS analysis/sorting and genomics (NGS, RNAseq etc) applications

REGULATORY STATUS

Research Use Only (RUO)

DESCRIPTION

BD Tumor Dissociation Reagent (Material Number 661563) has been designed to enable solid tumor tissue disaggregation into quality single cell suspension from fresh or overnight shipped xenografts and primary tumors from breast cancer specimens. The reagent has not been tested for FFPE tumor tissues. The dissociation reagent was optimized for viable cell yield of both tumor cells and immune infiltrates without any selection bias. This reagent has been formulated for better preservation of commonly used cell surface markers on tumor and infiltrating immune cells. The resulting single-cell suspension is suitable for downstream analysis to explore tumor heterogeneity and tumor microenvironment using flow cytometry, e.g. isolation of cell subpopulation(s) by FACS sorting followed by molecular analysis, i.e. gene expression.

STORAGE

Store dry at 2-8°C. Reagent is stable for 12 months after manufacture. See vial label for expiration date.

INSTRUCTIONS FOR USE

Reconstitute the contents of one vial in 5ml DMEM (Life Technologies, Cat. No. 11995073) to make a 2X stock; keep the reconstituted reagent at 37°C until ready to process the tumor sample and for no more than 30 minutes after reconstitution. This solution is enough to process one solid tumor tissue biopsy sample (0.05-1.0 grams).

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES

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PROCEDURE

This is standardized protocol designed to prepare high quality single cell suspensions from solid tumor biopsies from xenografts and breast cancer patients for downstream applications such as flow cytometry or genomics analysis.

Required materials

Material	Vender	Product ID#
DPBS (w/o Ca++/Mg++)	CellGro	21-031-CV
Pharm Lyse	BD	555899
BSA	Sigma Aldrich	A7906
DMEM	Life Technologies	11995073
EDTA (200 mM)	Fisher Scientific	50-841-657
50 mL conical centrifuge tube	VWR	89004-364
70 µm Cell Strainer	VWR	21008-952
1000 µl wide-orifice pipette tips	VWR	89049-168
Disposable Scalpels (blade #11)	VWR	21909-672
Glass Petri Dish 100 x 20 mm	VWR	89000-306

Reagent Preparation

1. Make a **1%BSA/DPBS/EDTA (no Ca++/Mg++)** solution by dissolving 10g BSA (Sigma Aldrich, Cat. No. A7906) and adding 10mL 200mM EDTA (Fisher Scientific, Cat. No. 50-841-657) into 1L DPBS without Ca++/Mg++ (CellGro, Cat. No. 21-031-CV). Sterile filter and store at 4°C. Discard after 1 week.
2. Make a **DPBS/EDTA (no Ca++/Mg++)** solution by adding 250mL of 200mM EDTA into 25mL DPBS (no Ca++/Mg++). Sterile filter and store at 4°C. Discard after 1 week.
3. Make **2x Dissociation Reagent:** On the day of tissue processing, prepare **2X Tumor Dissociation Reagent (TDR):** Add 5 mL of **DMEM** cell culture media to the brown amber vial containing **TDR**. Gently agitate periodically for 15 minutes at room temperature to ensure complete reconstitution of dried reagent. Transfer **TDR** to pre-labelled 50 mL conical centrifuge tube (VWR, Cat. No. 11995073). Discard amber vial. Add 30 mL DMEM to a freshly labeled 50 mL conical centrifuge tube. Place the conical tubes containing **2X TDR** in 35 mL of DMEM in a 37° C water bath (Do not leave the **TDR** in the water bath any longer than 30 minutes prior to use).

Unpack and weigh tissue samples

1. Unpack specimen(s) from shipping container and place on ice.
2. Add 5 mL of DMEM (warm; see DMEM prep in step 3 above) into fresh, labelled 100 x 20 mm glass petri dish (VWR, Cat. No. 89000-306) and set aside.
3. Place a weigh boat or other suitable receptacle to hold tissue during weighing and tare scale. Use tweezers to transfer tumor piece(s) from petri dish into weigh boat on scale (dab tissue specimen on paper towel to wick away excess transport buffer to ensure accurate weigh). After weighing the sample, **record weight of entire**

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tumor and return the tissue to the empty petri dish containing 5 mL of warm DMEM. Based on the weight of tumor, determine conditions for experiment. **It is recommended that 1 aliquot of dissociation reagent be used for no more than 1 gram of tissue. If your sample is larger than 1 gram, it may be necessary to split sample into multiple digestions.**

4. If multiple dissociation conditions are desired or if the tissue sample is greater than 1 gram in weight, the sample can be divided into multiple pieces and processed separately in parallel. If multiple digestions are required or desired, prepare any necessary additional petri dishes (label accordingly). Use disposable scalpel (VWR, Cat. No. 21909-672) and tweezers to cut tumor sample into roughly equal size pieces (the number of pieces will be determined by the number of digestion conditions required). If the tumor contains an obvious necrotic core, include an equal portion of this core in each of the subdivided pieces. Distribute the tumor pieces into each of the petri dishes. Weigh tissue in each petri dish (dab tissue on paper towel to wick away excess media prior to weighing to ensure accurate weight). Use no less than 50 mg of tissue for each condition. Record weight of each tumor piece.

Manually mince tissue

1. Using two scalpels to manually mince the tissue in the petri dish. The resulting tissue pieces should be as small as possible, with no pieces larger than 0.75 mm in diameter. Use pictures below as a reference. The picture on the left shows a tumor before mincing and the picture on the right shows the same tumor after mincing. **Mince the tissue as much as possible.**

Note: *Scalpels are an effective mincing method but other methods have been used with success such as dissection scissors in the bottom of a 15 mL conical tube. Use your discretion in choosing the best mincing method available*. However, be aware that inadequate mincing can significantly reduce cell yield.

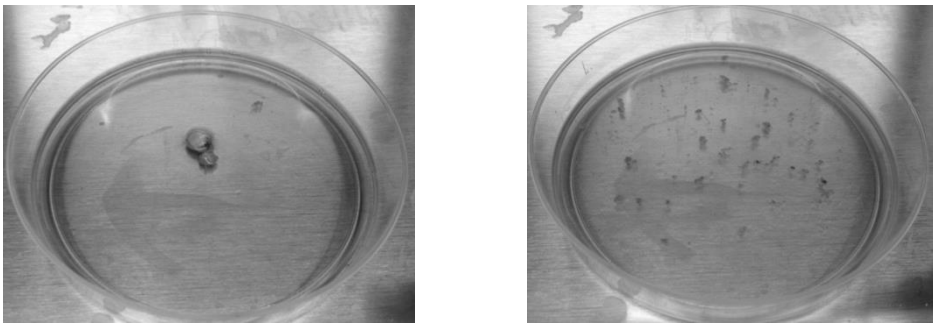

Figure 1: Use pictures as a reference. The picture on the left shows a tumor before mincing and the picture on the right shows the same tumor after mincing.

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2. Using 1000 µl wide-mouth pipette tips (VWR, Cat. No. 89049-168), transfer contents of each petri dish (media and tumor pieces) into a fresh, labelled 50 mL conical centrifuge tube. Place tube(s) on ice until all mincing is completed (any additional digestions). ***Note: If only processing one digestion, skip this step and transfer petri dish contents directly into 50 mL conical tube containing warm 2X TDR.***
3. Once all mincing has been completed, retrieve conical centrifuge tube(s) containing 5 mL of warm **2X TDR** from water bath. Using 1000 µl wide-mouth pipette tips, transfer contents of each of the conical tubes on ice (media and tumor pieces) into the conical centrifuge tube containing **2X TDR**. The final volume in each 50 mL conical should be 10 mL (5 mL warm TDR + 5 mL minced tumor in media).

Incubate digestion(s) 30 min at 37°C

1. Incubate digestions for 30 minutes at 37° C with mild but frequent agitation. This can be achieved using a shaking water bath or by placing a blood rocker inside an incubator. It is also possible to place the digestions in a rack inside the incubator and manually agitating the tubes by gentle inversion every 7-10 minutes.
2. After incubation, add 25 mL of PBS + 1% BSA + 2 mM EDTA to the 50 mL conical centrifuge tube(s) containing the dissociation reaction to bring total volume up to 35 mL. Pour contents of tube though a fresh 70 µm strainer (VWR, Cat. No. 21008-952) into a fresh, pre-labeled 50 mL conical tube. Rinse the strainer with an additional 10 mL of PBS + 1% BSA + 2 mM EDTA to collect residual single cells from the left over material (Total volume should be approximately 45 mL). Discard strainer and centrifuge the centrifuge tube at *250 x g* for 8 minutes.

Wash & count

1. Retrieve tube(s) from centrifuge and using a Pasteur pipette connected to a vacuum, remove supernatant. Gently resuspend pellets in 2 mL Pharm Lyse (BD Biosciences, Cat. No. 555899; before use, dilute 1:10 in water per manufacturer's instructions). Incubate for 15 minutes at room temperature and then add 40 mL PBS + 1% BSA + 2 mM EDTA. Centrifuge sample at *250 x g* for 8 minutes.
2. Use Pasteur pipette connected to a vacuum to remove supernatant completely ("dry pellet"). Resuspend each pellet in 2 mL PBS + 2 mM EDTA or to avoid clumping use Pre-Sort buffer (BD Biosciences, Cat. No. 563503).
3. Proceed with cell counts either by hemacytometer or automated cell counter. Record cell counts & viability.
4. Resulting cell-suspensions can be used for FACS analysis/sorting and other down-stream applications.

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REPRESENTATIVE DATA

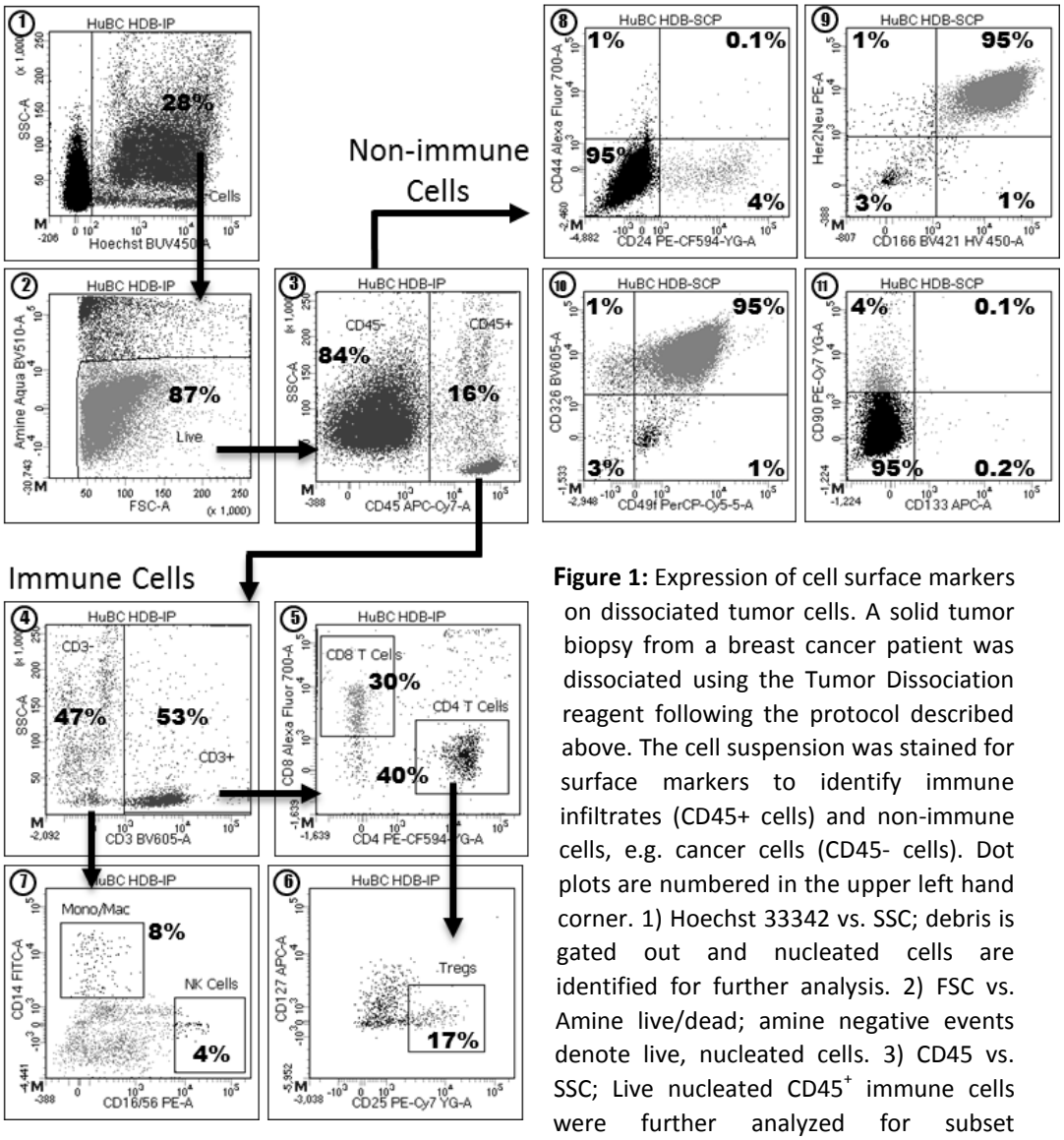

Figure 1: Expression of cell surface markers on dissociated tumor cells. A solid tumor biopsy from a breast cancer patient was dissociated using the Tumor Dissociation reagent following the protocol described above. The cell suspension was stained for surface markers to identify immune infiltrates (CD45+ cells) and non-immune cells, e.g. cancer cells (CD45- cells). Dot plots are numbered in the upper left hand corner. 1) Hoechst 33342 vs. SSC; debris is gated out and nucleated cells are identified for further analysis. 2) FSC vs. Amine live/dead; amine negative events denote live, nucleated cells. 3) CD45 vs. SSC; Live nucleated $CD45^{+}$ immune cells were further analyzed for subset composition (dot plots 4-7) and $CD45^{-}$ non-immune cells were further analyzed for expression of known cancer cell markers (dot plots 8-11). The samples were analyzed on SORP Fortessa cytometer using FACSDiva software.

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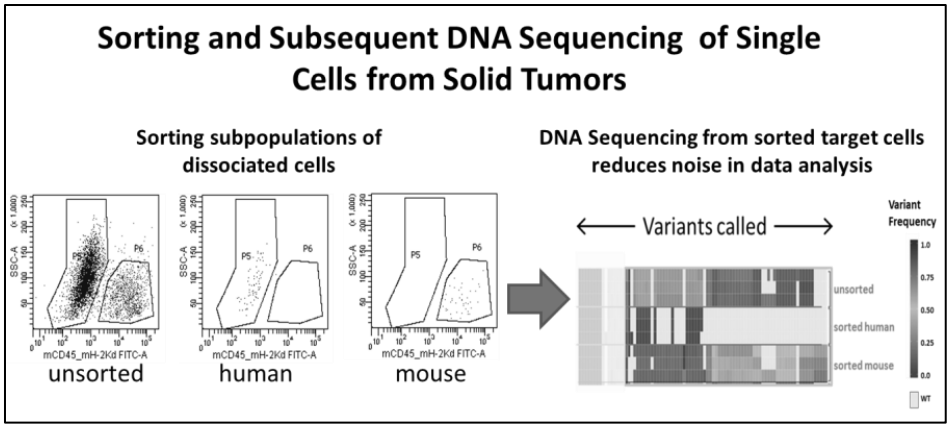

Figure 2: Xenograft breast tumors were dissociated into single-cell suspensions using the Tumor Dissociation Reagent following the protocol described above. Human tumor cells and mouse infiltrates were sorted based on species specificity as defined by expression of mouse CD45 + H-2kd. The purity of the sorted cell populations as measured by flow cytometry was >98%. Using the 2 sorted populations along with the unsorted to purify genomic DNA and further NGS sequencing using the Ion Torrent Cancer Hotspot panel v.2, a range of variants was detected with intermediate signal strength in the unsorted population, demonstrating that sorting of target cells prior to NGS analysis minimizes the noise due to contaminating cell populations.

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