

1. Technical Data Sheet

Summary	StimCytes™ CD20 Evaluation Kit synthetic cell mimics are lyophilized particles that feature selected biomarkers for use in CAR-T potency assays. This product contains no bio-hazardous material, so it is safe to use in any environment and requires no special disposal. For Research Use Only. Not for use in diagnostic or therapeutic procedures.
Application	Specific activation of CD20-targeted CAR-T for potency evaluation in co-culture assay with negative control.
Materials	StimCytes™ are hydrogels that are in lyophilized form. This product is lyophilized for stability and ease of use. StimCytes™ CD20 Evaluation Kit (SL-00043) is composed of two vials: 1. StimCytes™ CD20 with Enhancers (SL-00044) 2. StimCytes™ Negative with Enhancers (SL-00045) Each sealed glass vial contains 2M lyophilized particles.
Handling and Safety	No special handling or safety precautions are necessary.
Storage	Store lyophilized products at -20°C upon receipt.
Expiration	SL-00044: 12 months from the date of manufacturing when stored at -20°C. SL-00045: 24 months from the date of manufacturing when stored at -20°C. Use the entire vial immediately upon reconstitution of lyophilized product.

**Instructions
for Use**

1. Tap down the vial to ensure that all cell mimics are collected at the bottom of the vial.
2. Add 1000 μ L of media of choice to the vial and transfer contents to 15mL conical tube. Add additional 1000 μ L of media to vial, incubate 30 seconds to one minute, and transfer to same 15mL conical tube.
 - a. Note: Multiple vials can be combined into one 15mL conical.
 - b. Note: PBS with 1% BSA or RPMI with 10% FBS are recommended buffers for reconstitution. Please reach out to Slingshot Biosciences (support@slingshotbio.com) for alternate media suggestions.
3. Pellet cell mimics at 3000xg 3 mins at room temperature. Carefully remove supernatant from 15 mL tube with 1000 μ L pipette, or aspirate if mimic pellet is easily identifiable.
4. Resuspend pellet in 1000 μ L media per starting vial and repeat step 3.
5. Resuspend pellet in 1000 μ L media. Remove sample and count particles using a flow cytometer, hemacytometer, or automated counting instrument that incorporates phase contrast imaging to identify cells.
 - a. Note: If counts are performed on automated cell counter instrument with trypan blue exclusion, use total cell count. Automated cell counter instruments that rely exclusively on dye-based detection will require alternate methodology. Please reach out to Slingshot Biosciences (support@slingshotbio.com) for instrument-specific protocols or to discuss alternative options or custom solutions.
6. Pellet remaining sample as in (3), carefully remove supernatant, and resuspend mimics at desired concentration for assay.

QC Data	CD20-specific CAR-T activation at 1:1 E:T ratio for 24 hours <table border="1"><thead><tr><th>Activator</th><th>IFNγ (pg/mL)</th></tr></thead><tbody><tr><td>StimCytes™ CD20 with Enhancer(s)</td><td>~700</td></tr><tr><td>StimCytes™ Negative with Enhancer(s)</td><td>~50</td></tr><tr><td>RAJI</td><td>~6000</td></tr></tbody></table> Figure 1. Specific activation of CD20 CAR-T. CD20 CAR-T (BPS Biosciences) were cultured at 1:1 E:T with StimCytes™ Negative with Enhancers (SL-00045), StimCytes™ CD20 with Enhancers (SL-00044), or Raji cell line. Cultures were performed in U-bottom plates with RPMI 1640-based media supplemented with 10% FBS and other additives. Supernatant collected after 24 hours of culture was quantified for IFNγ with BD CBA on Cytex Aurora.	Activator	IFN γ (pg/mL)	StimCytes™ CD20 with Enhancer(s)	~700	StimCytes™ Negative with Enhancer(s)	~50	RAJI	~6000
Activator	IFN γ (pg/mL)								
StimCytes™ CD20 with Enhancer(s)	~700								
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Technical Support	For technical support from our Cell Therapy scientists, please contact support@slingshotbio.com Individual results may vary. Slingshot Biosciences Cell Therapy scientists are available for technical support and suggestions for customization to achieve optimal results.								