

Barcoded Antibodies spike-in Pixelgen Proxiome Kit v2

Demonstrated protocol

This protocol describes how to spike in additional **Barcoded Antibodies** that are not included in the standard **Antibody Panel** of the Pixelgen Proxiome Kit v2. Perform this protocol before starting **STEP 3 - Barcoded Antibody Binding** in UM002: User Manual Proxiome Kit v2 Cell Surface (v1.00). Additional antibodies may be included as either **CAR T Barcoded Antibodies** supplied by Pixelgen and/or as **Custom Barcoded Antibodies** prepared using the Pixelgen Proxiome v2 Custom Conjugation Kit. Depending on the combination used, follow the applicable situation described below.

NOTE: Up to two **CAR T Barcoded Antibodies** may be selected from the three available options listed below. In addition, up to two **Custom Barcoded Antibodies** may be prepared using the Pixelgen Proxiome v2 Custom Conjugation Kit. The applicable protocol depends on whether the spike-in consists of **CAR T Barcoded Antibodies** only, **Custom Barcoded Antibodies** only, or a combination of both. Table 1 summarizes the different possible spike-in setups.

Table 1. Spike-in setups

Spike-in setup	Situation
1-2 CAR T Barcoded Antibodies only	Situation 1
1-2 Custom Barcoded Antibodies only	Situation 2
1-2 CAR T Barcoded Antibodies + 1-2 Custom Barcoded Antibodies	Situation 3

Three **CAR T Barcoded Antibodies** are available:

1. FMC63 (Product No. PP102)
2. FLAG (Product No. PP103)
3. G4S (Product No. PP104)

The volume of spike-in mix to add to the **Antibody Panel** depends on the Pixelgen Proxiome Kit v2 configuration, as detailed in Table 2 below.

Table 2. Spike-in volume to add into the **Antibody Panel** depending on the Pixelgen Proxiome Kit v2 configuration

Pixelgen Proxiome Kit v2 configuration	Volume of prepared spike-in mix (μ l)
8-reaction kit	4.4
16-reaction kit	6.4
32-reaction kit	10.4

SITUATION 1 - Spike-in of CAR T Barcoded Antibodies only

NOTE: The spike-in instructions differ depending on whether one or two CAR T Barcoded Antibodies are used.

- A. Mix each **CAR T Barcoded Antibody** by pipetting up and down 5 times.
- B. Prepare the CAR T Barcoded Antibody spike-in pool as follows:
 - a. If using one **CAR T Barcoded Antibody**, mix 7 μ l of the **CAR T Barcoded Antibody** with 14 μ l of **1x PBS** (total of 21 μ l).
 - b. If using two **CAR T Barcoded Antibodies**, mix 7 μ l of the **CAR T Barcoded Antibody 1** with 7 μ l of the **CAR T Barcoded Antibody 2** and with 7 μ l of **1x PBS** (total of 21 μ l).
- C. Add the prepared CAR T Barcoded Antibody spike-in pool to the **Antibody Panel** according to Table 2 above. Store the remaining spike-in pool at +4°C up to 12 months. **Do not freeze.**

NOTE: Please note that the volume to add depends on the Pixelgen Proxiome Kit v2 configuration.

SITUATION 2 - Spike-in of Custom Barcoded Antibodies only

NOTE: The Pixelgen Proxiome v2 Custom Conjugation Kit allows conjugation of up to two antibodies of your choice. A unique barcode is assigned to each antibody, and each barcode is available in two versions: Oligo A and Oligo B. Please refer to UM003: Pixelgen Proxiome v2 Custom Conjugation Kit (v1.00) for instructions.

- A. Mix both versions of each **Custom Barcoded Antibody** (i.e., Oligo A and Oligo B) by pipetting up and down 5 times.
- B. Prepare the Custom Barcoded Antibody spike-in pool as follows:
 - a. If using one Custom Barcoded Antibody:

Component	Volume to add
Custom Barcoded Antibody 1 - Oligo A	2.5 μ l
Custom Barcoded Antibody 1 - Oligo B	2.5 μ l
Storage Solution	40 μ l
1x PBS	55 μ l
Total volume	100 μl

- b. If using two Custom Barcoded Antibodies:

Component	Volume to add
Custom Barcoded Antibody 1 - Oligo A	2.5 µl
Custom Barcoded Antibody 1 - Oligo B	2.5 µl
Custom Barcoded Antibody 2 - Oligo A	2.5 µl
Custom Barcoded Antibody 2 - Oligo B	2.5 µl
Storage Solution	40 µl
1x PBS	50 µl
Total volume	100 µl

- C. Add the prepared Custom Barcoded Antibody spike-in pool to the **Antibody Panel** according to Table 2 above. Store the remaining spike-in pool at +4°C up to 12 months. **Do not freeze.**

SITUATION 3 - Spike-in of CAR T Barcoded Antibodies and Custom Barcoded Antibodies

- A. Mix both versions of each **Custom Barcoded Antibody** (i.e., Oligo A and Oligo B), and mix each **CAR T Barcoded Antibody**, by pipetting up and down 5 times.
- B. Prepare the spike-in pools as follows:
- a. If using one **CAR T Barcoded Antibody** and one **Custom Barcoded Antibody**
- i. Prepare the following mix:

Component	Volume to add
Custom Barcoded Antibody 1 - Oligo A	7.5 µl
Custom Barcoded Antibody 1 - Oligo B	7.5 µl
Storage Solution	40 µl
1x PBS	45 µl
Total volume	100 µl

- ii. Mix 7 μ l of the **CAR T Barcoded Antibody** with 7 μ l of the prepared spike-in pool of the **Custom Barcoded Antibody** and with 7 μ l of **1x PBS** (total of 21 μ l).
- iii. Add the prepared spike-in pool to the **Antibody Panel** according to Table 2 above. Store the remaining spike-in pool at +4°C up to 12 months. **Do not freeze.**

b. If using one **CAR T Barcoded Antibody** and two **Custom Barcoded Antibodies**:

- i. Prepare the following mix:

Component	Volume to add
Custom Barcoded Antibody 1 - Oligo A	7.5 μ l
Custom Barcoded Antibody 1 - Oligo B	7.5 μ l
Custom Barcoded Antibody 2 - Oligo A	7.5 μ l
Custom Barcoded Antibody 2 - Oligo B	7.5 μ l
Storage Solution	40 μ l
1x PBS	30 μ l
Total volume	100 μl

- ii. Mix 7 μ l of the **CAR T Barcoded Antibody** with 7 μ l of the prepared spike-in pool of the **Custom Barcoded Antibodies** and with 7 μ l of **1x PBS** (total of 21 μ l).
- iii. Add the prepared spike-in pool to the **Antibody Panel** according to Table 2 above. Store the remaining spike-in pool at +4°C up to 12 months. **Do not freeze.**

c. If using two **CAR T Barcoded Antibodies** and one **Custom Barcoded Antibody**:

- i. Prepare the following mix:

Component	Volume to add
Custom Barcoded Antibody 1 - Oligo A	7.5 μ l
Custom Barcoded Antibody 1 - Oligo B	7.5 μ l
Storage Solution	40 μ l
1x PBS	45 μ l
Total volume	100 μl

- ii. Mix 7 μ l of the **CAR T Barcoded Antibody 1** with 7 μ l of the **CAR T Barcoded Antibody 2** and with 7 μ l of the prepared spike-in pool of the **Custom Barcoded Antibody** (total of 21 μ l).
- iii. Add the prepared spike-in pool to the **Antibody Panel** according to Table 2 above. Store the remaining spike-in pool at +4°C up to 12 months. **Do not freeze.**

d. If using two **CAR T Barcoded Antibodies** and two **Custom Barcoded Antibodies**:

- i. Prepare the following mix:

Component	Volume to add
Custom Barcoded Antibody 1 - Oligo A	7.5 μ l
Custom Barcoded Antibody 1 - Oligo B	7.5 μ l
Custom Barcoded Antibody 2 - Oligo A	7.5 μ l
Custom Barcoded Antibody 2 - Oligo B	7.5 μ l
Storage Solution	40 μ l
1x PBS	30 μ l
Total volume	100 μl

- ii. Mix 7 μ l of the **CAR T Barcoded Antibody 1** with 7 μ l of the **CAR T Barcoded Antibody 2** and with 7 μ l of the prepared spike-in pool of the **Custom Barcoded Antibodies** (total of 21 μ l).
- iii. Add the prepared spike-in pool to the **Antibody Panel** according to Table 2 above. Store the remaining spike-in pool at +4°C up to 12 months. **Do not freeze.**

Please note that a different panel file is required when processing your samples with the Pixelator software. Please contact us at support@pixelgen.com if you have any questions.