Barcoded Antibodies spike-in Pixelgen Proxiome Kit v2

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. Additional antibodies may be included as either **CAR 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 Barcoded Antibodies** may be selected from the four 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 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 Barcoded Antibodies only	Situation 1
1-2 Custom Barcoded Antibodies only	Situation 2
1-2 CAR Barcoded Antibodies + 1-2 Custom Barcoded Antibodies	Situation 3

Four **CAR Barcoded Antibodies** are available:

- FMC63 (Catalog number CAR102)
- FLAG (Catalog number CAR103)
- G4S (Catalog number CAR104)
- Whitlow (Catalog number CAR105)

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 (µl)
8-reaction kit	4.4
16-reaction kit	6.4
32-reaction kit	10.4

SITUATION 1 - Spike-in of CAR Barcoded Antibodies only

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

A. ☐ Mix each **CAR Barcoded Antibody** by pipetting up and down 5 times.

B. ☐ Prepare the CAR Barcoded Antibody spike-in pool as follows:

a. If using one **CAR Barcoded Antibody**, mix 7 µl of the **CAR Barcoded Antibody** with 14 µl of **1x PBS** (total of 21 µl).

b. If using two **CAR Barcoded Antibodies**, mix 7 µl of the **CAR Barcoded Antibody 1** with 7 µl of the **CAR Barcoded Antibody 2** and with 7 µl of **1x PBS** (total of 21 µl).

C. ☐ Add the prepared CAR Barcoded Antibody spike-in pool to the **Antibody Panel** according to the Pixelgen Proxiome Kit v2 configuration, as indicated in Table 2 above. Store the remaining spike-in pool at 4°C up to 12 months. **Do not freeze**.

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: User Manual Pixelgen Proxiome v2 Custom Conjugation Kit 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 µl
Custom Barcoded Antibody 1 - Oligo B	2.5 µl
Storage Solution	40 µl
1x PBS	55 µl
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 the Pixelgen Proxiome Kit v2 configuration, as indicated in 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 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 Barcoded Antibody**, by pipetting up and down 5 times.

B. ☐ Prepare the spike-in pools as follows:

a. If using one **CAR 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 µl of the **CAR Barcoded Antibody** with 7 µl of the prepared spike-in pool of the **Custom Barcoded Antibody** and with 7 µl of **1x PBS** (total of 21 µl).

iii. Add the prepared spike-in pool to the **Antibody Panel** according to the Pixelgen Proxiome Kit v2 configuration, as indicated in Table 2 above. Store the remaining spike-in pool at 4°C up to 12 months. **Do not freeze**.

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

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
Custom Barcoded Antibody 2 - Oligo A	7.5 µl
Custom Barcoded Antibody 2 - Oligo B	7.5 µl
Storage Solution	40 µl
1x PBS	30 µl
Total volume	**100 µl**

ii. Mix 7 µl of the **CAR Barcoded Antibody** with 7 µl of the prepared spike-in pool of the **Custom Barcoded Antibodies** and with 7 µl of **1x PBS** (total of 21 µl).

iii. Add the prepared spike-in pool to the **Antibody Panel** according to the Pixelgen Proxiome Kit v2 configuration, as indicated in Table 2 above. Store the remaining spike-in pool at 4°C up to 12 months. **Do not freeze**.

c. If using two **CAR Barcoded Antibodies** 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 µl of the **CAR Barcoded Antibody 1** with 7 µl of the **CAR Barcoded Antibody 2** and with 7 µl of the prepared spike-in pool of the **Custom Barcoded Antibody** (total of 21 µl).

iii. Add the prepared spike-in pool to the **Antibody Panel** according to the Pixelgen Proxiome Kit v2 configuration, as indicated in Table 2 above. Store the remaining spike-in pool at 4°C up to 12 months. **Do not freeze**.

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

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
Custom Barcoded Antibody 2 - Oligo A	7.5 µl
Custom Barcoded Antibody 2 - Oligo B	7.5 µl
Storage Solution	40 µl
1x PBS	30 µl
Total volume	**100 µl**

ii. Mix 7 µl of the **CAR Barcoded Antibody 1** with 7 µl of the **CAR Barcoded Antibody 2** and with 7 µl of the prepared spike-in pool of the **Custom Barcoded Antibodies** (total of 21 µl).

iii. Add the prepared spike-in pool to the **Antibody Panel** according to the Pixelgen Proxiome Kit v2 configuration, as indicated in 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.