BioXp® Select plasmid amplification kit — Loading map checklist and kit information

BioXp Select plasmid amplification kit includes Module A (Room Temp), Module J (Room Temp), and Module I (−80°C).

Prepare DNA input plate on the bench

Prepare the reagents on decontaminated benchtop with clean pipettes and sterile pipette tips. Avoiding contamination is critical for the selective amplification of target DNA. We recommend cleaning bench and pipettes with cleaning solution that is meant to remove contaminants of PCR reactions (PCR Clean Wipes, Minerva Biolabs, Cat. No.:15-2001 or similar).

1. Prepare the DNA input plate with your template plasmids to be amplified. The number of templates to be amplified is dictated by the kit type and therefore the number of reactions.
   - Dilute the plasmid DNA to 2-20 ng/µL in nuclease-free water. See user guide for more guidelines on template preparation.
   - Carefully pipette 35 µL of each diluted plasmid into the DNA input plate according to Table 1 below. The positive control plasmid (pUC19) provided in Module I can be loaded into a well to ensure proper kit function. Refer to the user guide for more information about the pUC19 sequence.
   - Spin plate briefly to collect the liquid at the bottom of the wells and to eliminate bubbles in the wells.
   - Maintain the DNA input plate on ice until the start of the run.

Number of reactions	Job Type*	DNA template input plate (template numbers, n-m)
8	S.A.8	A01-H01 (1-8)
16	S.A.16	A01-H01 (1-8) A02-H02 (9-16)
24	S.A.24	A01-H01 (1-8) A02-H02 (9-16) A03-H03 (17-24)

Table 1. DNA input plate preparation instructions for each job type

*Job Type code is displayed on the black metal biosecurity cover

2. Thaw and spin the DNA amplification strip.
   - Thaw the DNA amplification strip (stored at −80 °C) on ice for 30 to 60 minutes. Alternatively, strips can be thawed at room temperature for 10 minutes, then placed on ice for up to an hour until use.
   - Briefly spin the DNA amplification strip to remove bubbles and collect the liquid at the bottom of the tube.

Prepare and load the BioXp system deck

Refer to the instructions and image of the deck **(Fig. 1)**. Ensure that all kit components are properly prepared and placed before starting the run. See **Table 2** for a list of reagents and parts included with each kit.

Cleaning and preparing the BioXp 3250 system

3. Empty the waste bin in position 8 and clear the BioXp system of any used tips and plates.
4. Spray 70% Ethanol onto a lint-free wipe and decontaminate the exposed BioXp system surfaces. Wipe the four pipettors of the pipette head. Do not spray directly into the BioXp system.
5. To further decontaminate the system, repeat the cleaning process with a cleaning solution that is meant to remove contaminants of PCR reactions (PCR Clean Wipes, Minerva Biolabs, Cat. No.:15-2001 or similar).
6. Ensure the thermal cover is in position 1 before beginning.

Loading plates and reagents into the BioXp 3250 system

7. Load the empty Reaction plate into **position 2**. Ensure that the white plate carrier and the black metal plate cover are both present. *For proper system initialization, ensure the plate cover is oriented so the job type and corresponding QR code are facing you.*
8. Ensure the ethanol reservoir is empty of liquid and load into **position 3**. Although empty, the ethanol reservoir must be present for proper system initialization.
   - Do not discard the ethanol reservoir after the run; keep for future use.

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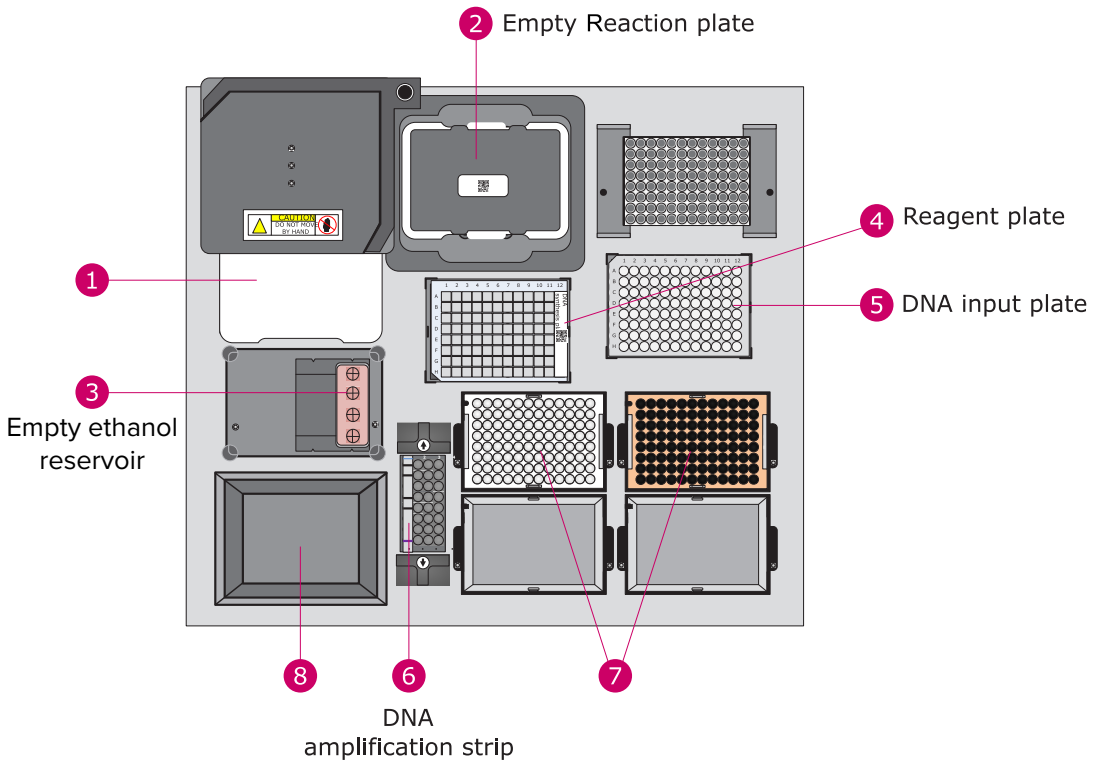
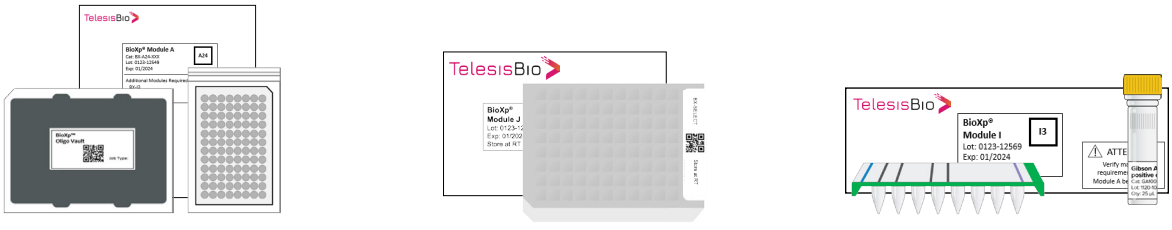


Figure 1. Layout of the BioXp deck with components and their corresponding positions, labeled with pink circles. Component positions are: (1) Thermal cover, (2) Reaction plate (an empty 96-well reaction plate in a white carrier covered by a metal biosecurity cover, from Module A), (3) empty ethanol reservoir, (4) Reagent plate, or Module J, (5) DNA input plate from Module A, (6) Reagent strip location (for DNA Amplification Strip from Module I; see Fig. 3 for specifics), (7) Pipette tip trays (See Fig. 4 for specifics), and (8) waste bin.

# of reactions	Job Type code	Kit SKU	Reagent plate (Module J)	DNA Amplification strip (Module I)	50 µl tip trays	200 µl tip trays
8	S.A.8	BX-SEL-PLSAMP-08	BX-SELECT	BX1350-08	1	1
16	S.A.16	BX-SEL-PLSAMP-16	BX-SELECT	BX1350-16	1	1
24	S.A.24	BX-SEL-PLSAMP-24	BX-SELECT	BX1350-24	1	1

Table 2. Reagents and parts included with each kit.



Module A Module J Module I

Figure 2. Images of kit components contained in each kit. Components are supplied as Modules A, J, and I. Module A contains the DNA input plate and the Reaction plate (with carrier and metal biosecurity cover), Module J contains the Reagent plate, and Module I contains the DNA amplification strip, and the positive control plasmid (pUC19).

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9. Load the Reagent plate into **position 4**.
   - Ensure the label is on the right-hand side of the plate and that the notch in the plate plastic is in the lower left corner. Make sure the plate is secured flush with the plate holder.
10. Carefully load the DNA input plate into **position 5**.
    - Refer to Step 1 above for more information about how to prepare the DNA input plate with your templates.
    - Take care so that liquid is not dislodged from the bottom of the plate wells. If liquid is dislodged, spin the plate again and re-load.
11. Carefully load and secure the DNA amplification strip into **position 6**. *For proper system initialization, secure strip with the spring-loaded arms while holding the strip securely in place.*
    - The DNA amplification strip should always be in the left-most strip holder. Ensure that the pattern of stripes printed on the strip foil matches what is shown in Fig. 3 below.
    - The other 3 strip holders remain empty.

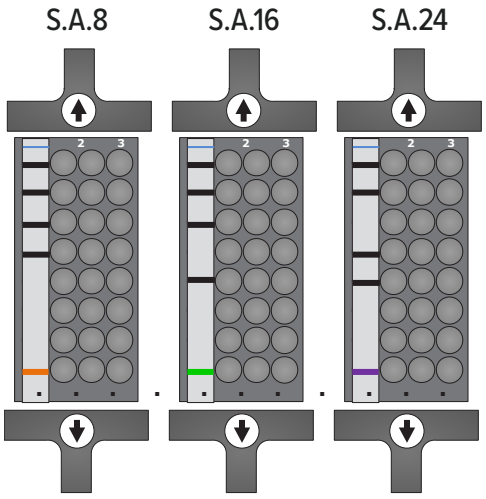

Figure 3. DNA amplification strip configuration in **position 6**. Shown are the positions and striping colors/ patterns corresponding with each job type. The strip position is the same for all kit sizes.

12. Load fresh (unopened) tips by aligning the tip tray notch with the upper left corner of each tip tray retainer. The tip loading is the same for all kit sizes. See **Fig. 4** below for more information.
    - Load 1 × 50 µL tips to the upper left-hand tray 1.

    Note: TipOne Clear (Part No. 1181-5700) or Tecan Clear (Part No. 30126096) tips can be used.
    - Load 1× 200 µL tips to the upper right-hand tray 2.

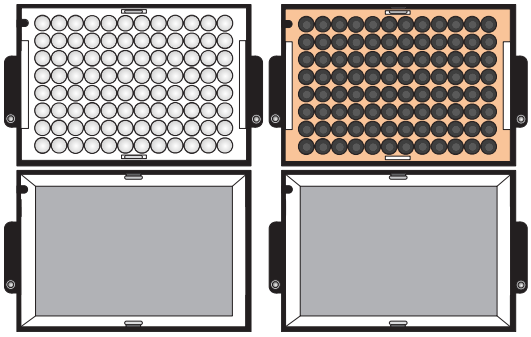

Figure 4. Pipette tip configuration in position 7. Shown are the positions of the tips. This tip configuration is valid for all Select plasmid amplification job types (S.A.8, S.A.16, S.A.24).

13. Once all components are loaded, confirm that all components are securely seated. Refer to **Fig. 1** to check the deck loading.
14. Close the door to initialize the job and follow the software prompts. The BioXp system will inspect the deck.
15. After the deck inspection, press Start to begin the run.

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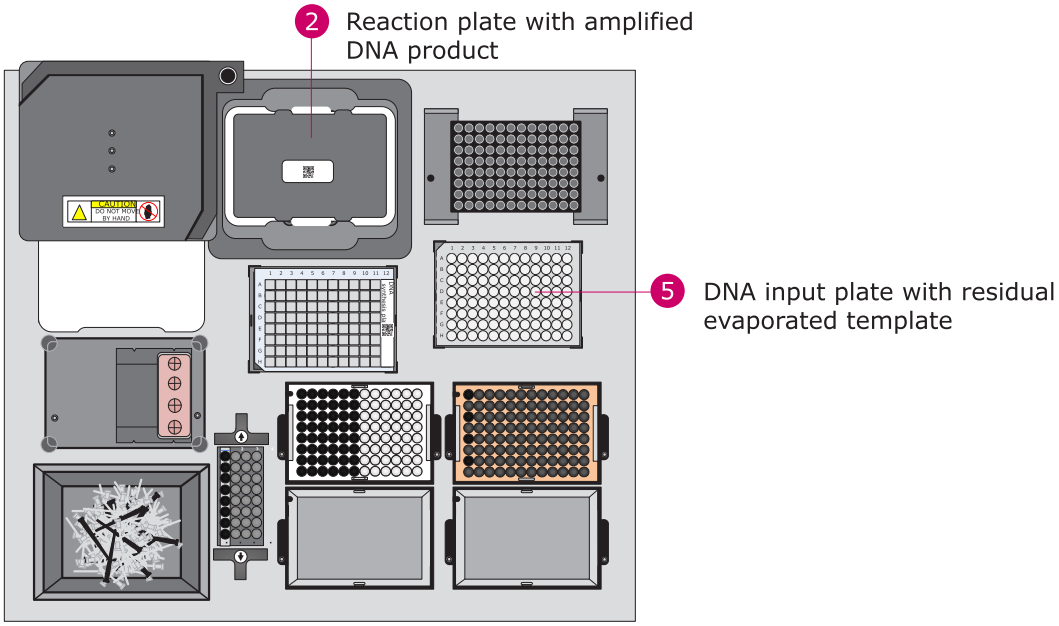

Figure 5. BioXp deck at the end of a run. The 24-well run (S.A.24) is shown as an example. Note that plates do not change locations in this job. Collect the reaction plate from position 2 with amplified DNA concatemer product for processing. The DNA input plate can be discarded.

Finishing the run and collecting reaction products

Fig. 5 shows deck configuration upon completion of successful run. At completion, save the Reaction Plate. DNA Input Plate, Reagent Plate, spent DNA Amplification Strips, and unused tips can be discarded. Remember to save the Ethanol reservoir for future runs.

The Reaction Plate is in the thermal cycler **(position 2)** at the end of a run. This plate contains the amplified DNA concatemer products. Seal the plate before removing from the white carrier to avoid dislodging the sample from the wells. Store the sealed reaction plate at –20 °C for up to six months. **Note that pipetting of the viscous DNA amplification product will result in irregular pipetting and incomplete volume transfer. Proceed with restriction digest of the product in the Reaction plate to ensure accurate pipetting.**

The table below details the addresses of the templates in the DNA input plate, and the corresponding amplification products in the reaction plate

Number of wells	Job type	DNA input plate well locations	Reaction plate well locations
8	S.A.8	A01-H01 (1-8)	A02-H02 (1-8)
16	S.A.16	A01-H01 (1-8) A02-H02 (9-16)	A02-H02 (1-8) A03-H03 (9-16)
24	S.A.24	A01-H01 (1-8) A02-H02 (9-16) A03-H03 (17-24)	A02-H02 (1-8) A03-H03 (9-16) A04-H04 (17-24)

Table 3. Templates in the DNA input plate, and the corresponding amplification products in the reaction plate.

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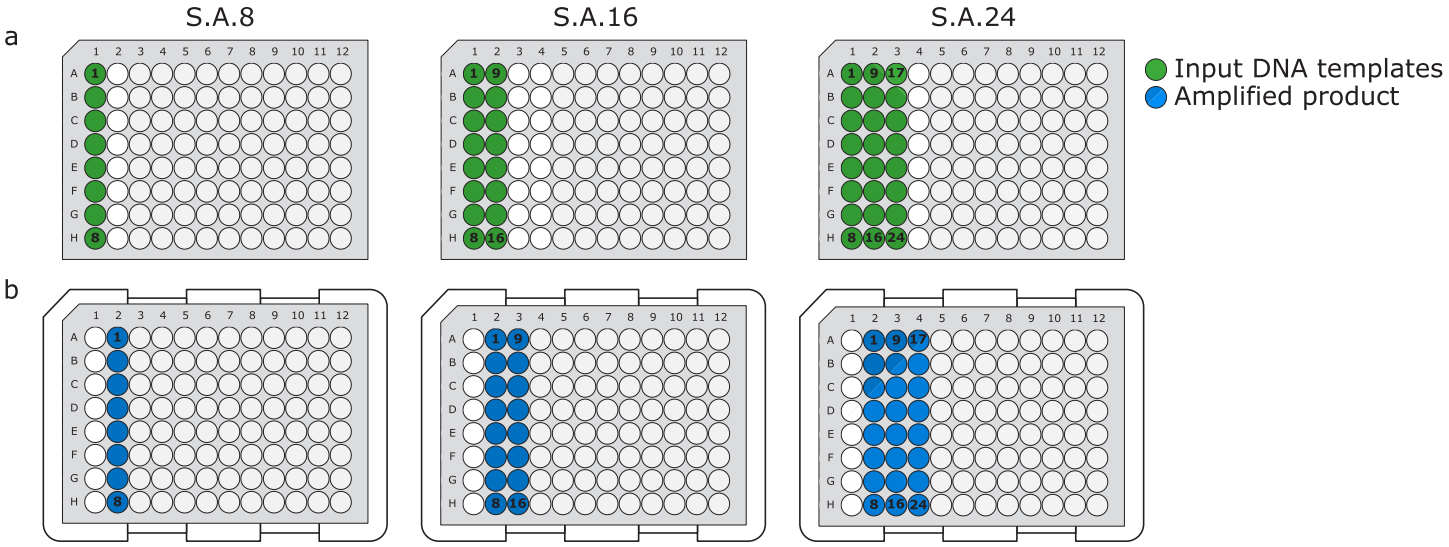

Figure 6. Templates and amplification products for each kit type. Shown are (a) the well addresses of input DNA templates loaded into the DNA input plate, and (b) the corresponding well addresses of the amplified DNA concatemer product, found in the Reaction plate. Note: amplified products are offset by one column to the right with respect to the input locations.

For technical assistance, contact help@telesisbio.com.

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