TrueMark™ Prostate Protein Assay

Catalog Number A46360

Pub. No. MAN0019000 Rev. A.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *TrueMark™ Prostate Protein Assay User Guide* (Pub. No. MAN0017766). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This Quick Reference is intended as a benchtop reference for experienced users of the TrueMark™ Prostate Protein Assay. For detailed instructions, supplemental procedures, and troubleshooting, see the *TrueMark™ Prostate Protein Assay User Guide* (Pub. No. MAN0017766).

Assay procedure: Day 1

Prepare the Sample Stocks Plate

EDTA plasma samples

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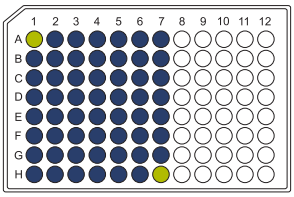
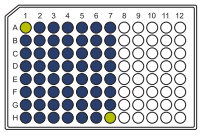
Manually transfer aliquots of plasma test samples (●) plus the TrueMark™ Prostate Positive Control (●) to a Fast 96-well plate



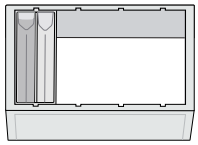
Sample Stocks Plate

Set up, then run the Plate 1 binding reactions on the Biomek™ FX^P instrument

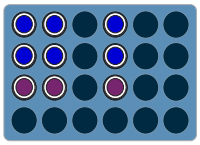
Plate 1 binding reactions (KLK2, PSP94)



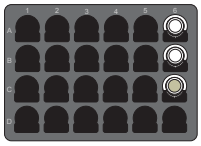
Sample Stocks Plate



Sample Buffer



Probes (left) and **Calibrators** (right) (● KLK2, ● PSP94)



○ **2 × Probe Buffer,** ● **PSP94 Buffer**

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Ensure that all reagents, plates, samples, and tips are in the correct location on the instrument deck (see Figure 1)

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Run Binding Reaction Setup Method—Plate 1 (~50 minutes)

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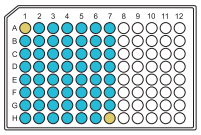

Sample Dilutions Plate

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Proceed to set up Plate 2 binding reactions

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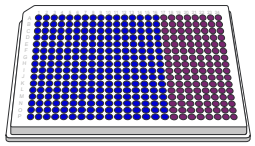


Plate 1 (samples + Probes + Calibrators)

▼

>16 hours, 4°C (binding reaction)

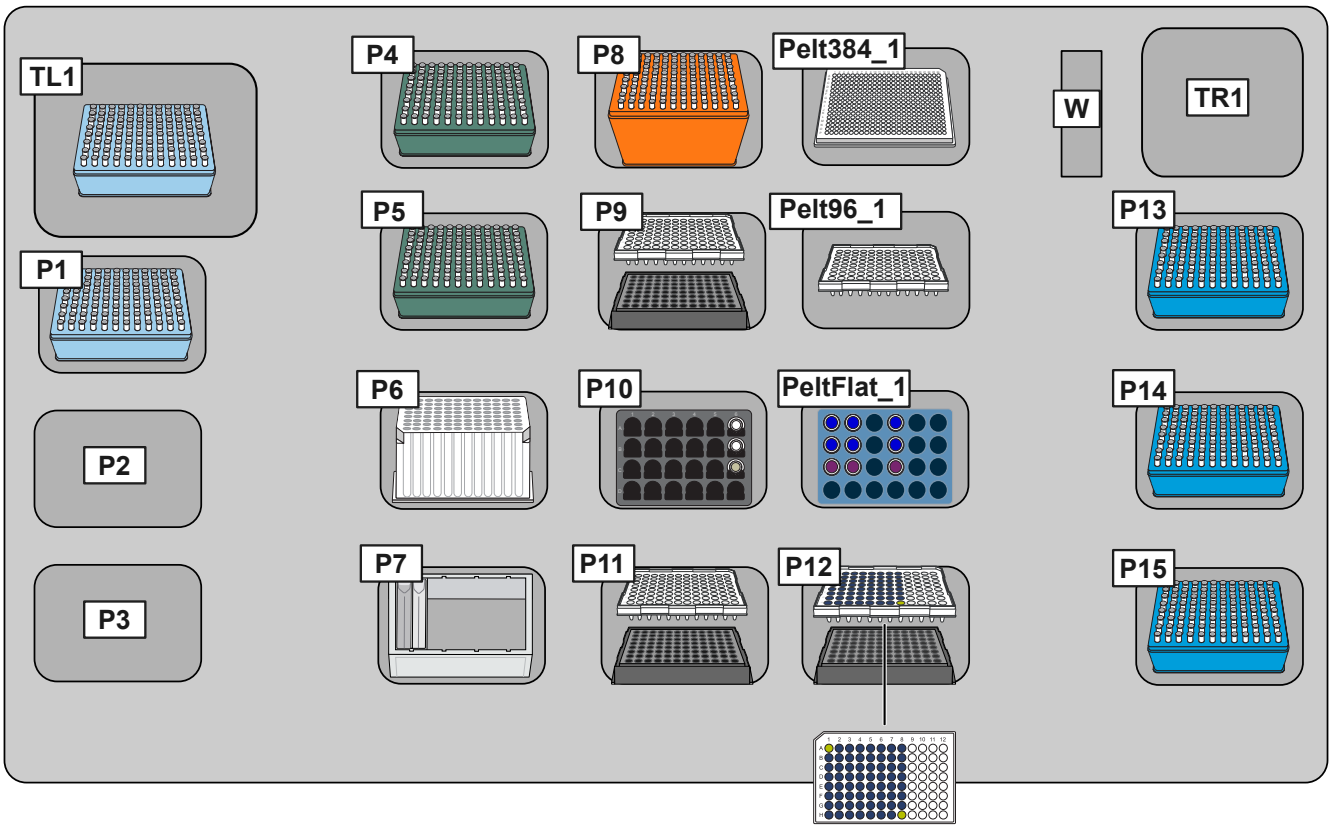


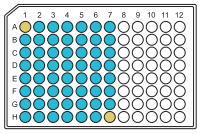
Fig. 1 Biomek™ FX^P deck setup for Binding Reaction Setup Method—Plate 1

TL1	AP96 P20 Tips
P1	AP96 P20 Tips
P4	Span-8 P250 Tips
P5	Span-8 P250 Tips
P6	1.0-mL deep-well plate
P7	Sample Buffer (quarter reservoir in frame, liquid on the left)
P8	Span-8 P1000 Tips
P9	Fast 96-well plate on 96-well base
P10	2-mL rack (2 × Probe Buffer, PSP94 Buffer)
P11	*Empty* Sample Dilutions Plate (Fast 96-well plate) on 96-well base
Pelt384_1	*Empty* Plate 1 (384-well EnduraPlate™)
Pelt96_1	Fast 96-well plate
PeltFlat_1	0.5-mL rack (Probes A and B, Calibrators for KLK2, PSP94)
P12	Sample Stocks Plate on 96-well base
W	Liquid waste
TR1	Tip waste
P13	Span-8 P20 Tips
P14	Span-8 P20 Tips
P15	Span-8 P20 Tips

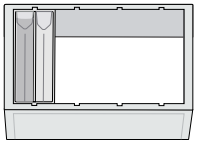
Set up, then run the Plate 2 binding reactions on the Biomek™ FX^P instrument

Plate 2 binding reactions (FPSA, PSA, GDF15)

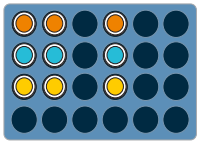
(start after Binding Reaction Setup Method—Plate 1 is complete)



Sample Dilutions Plate

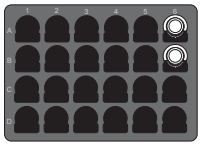


Sample Buffer



Probes (left) and **Calibrators** (right)

(FPSA, PSA, GDF15)



2 × Probe Buffer

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Ensure that all reagents, plates, samples, and tips are in the correct location on the instrument deck (see Figure 2)

▼

Run Binding Reaction Setup Method—Plate 2 (~45 minutes)

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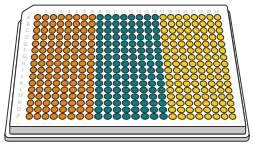


Plate 2 (samples + Probes + Calibrators)

▼

>16 hours, 4°C (binding reaction)

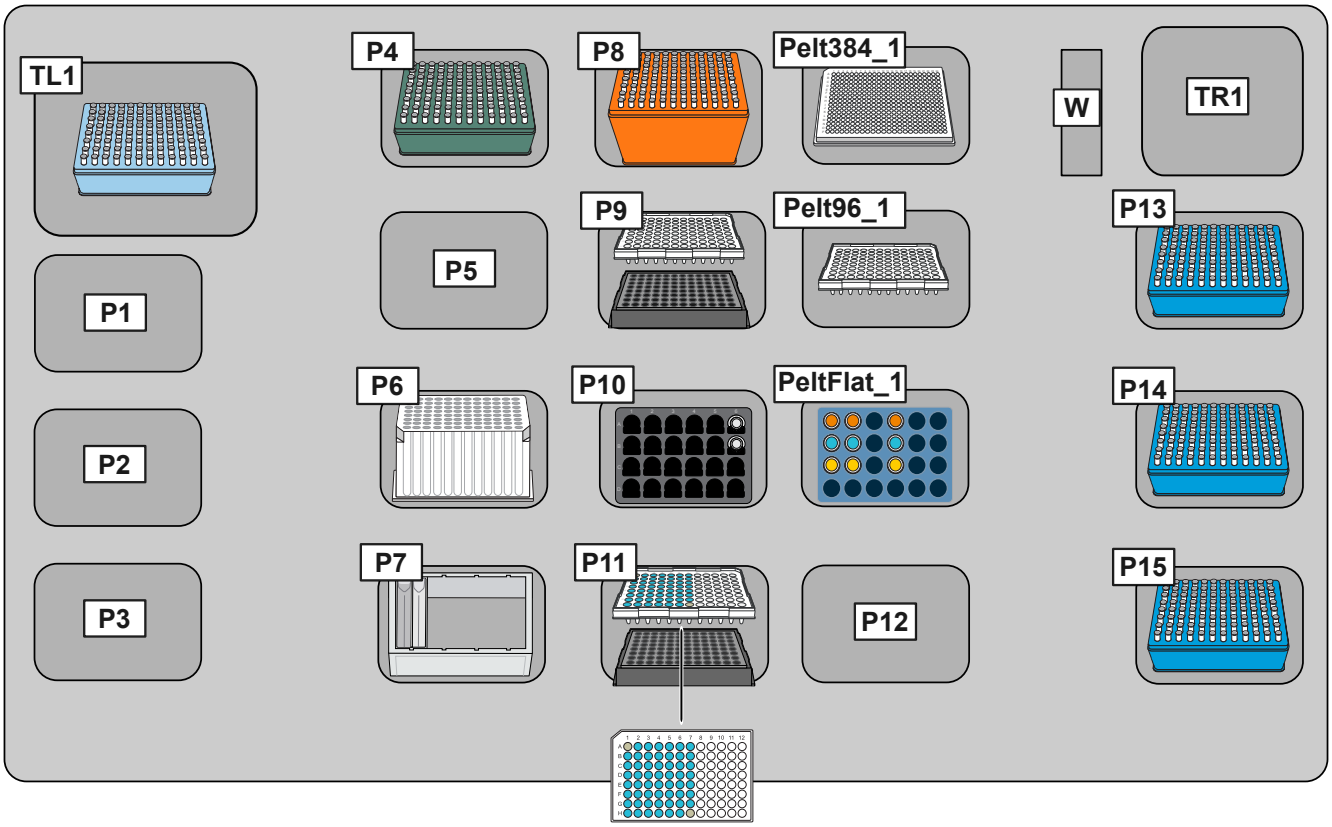


Fig. 2 Biomek™ FX^P deck setup for Binding Reaction Setup Method—Plate 2

TL1	AP96 P20 Tips
P4	Span-8 P250 Tips
P6	1.0-mL deep-well plate
P7	Sample Buffer (quarter reservoir in frame, liquid on the left)
P8	Span-8 P1000 Tips
P9	Fast 96-well plate on 96-well base
P10	2-mL rack (2 × Probe Buffer)
P11	Sample Dilutions Plate on 96-well base
Pelt384_1	Plate 2 (*empty* 384-well EnduraPlate™)
Pelt96_1	Fast 96-well plate
PeltFlat_1	0.5-mL rack (Probes A and B, Calibrators for FPSA, PSA, GDF15)
W	Liquid waste
TR1	Tip waste
P13	Span-8 P20 Tips
P14	Span-8 P20 Tips
P15	Span-8 P20 Tips

Assay procedure: Day 2

Set up, then run Ligation Reaction Setup Method on the Biomek™ FX^P instrument

Perform this procedure for Plate 1, then repeat for Plate 2.

Plate 1 (KLK2, PSP94)

IMPORTANT! Keep Plate 2 at 4°C during Plate 1 ligation and real-time PCR.

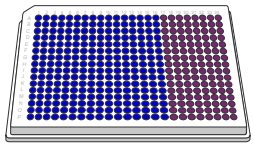
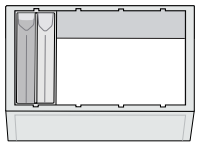
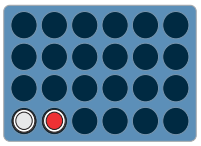


Plate 1
(probe binding to Plate 1 samples complete)



Ligation-qPCR Master Mix



DNA Ligase, Master Mix Additive

▼

Ensure that all reagents, plates, samples, and tips are in the correct location on the instrument deck (see Figure 3)

▼

Run Ligation Reaction Setup Method (~16 minutes)

▼

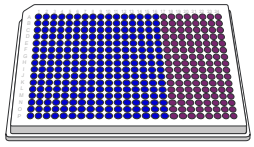


Plate 1 (ligation reaction complete)

▼

Proceed to “Run the ligation reaction on the ProFlex™ thermal cycler” on page 6

Plate 2 (FPSA, PSA, GDF15)

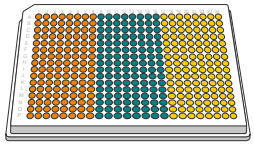
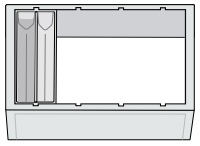
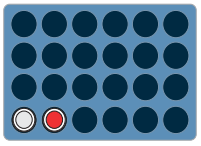


Plate 2
(probe binding to Plate 2 samples complete)



Ligation-qPCR Master Mix



DNA Ligase, Master Mix Additive

Ensure that all reagents, plates, samples, and tips are in the correct location on the instrument deck (see Figure 3)

▼

Run Ligation Reaction Setup Method (~16 minutes)

▼

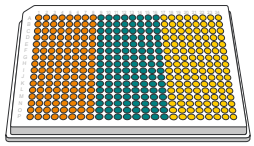


Plate 2 (ligation reaction complete)

Proceed to “Run the ligation reaction on the ProFlex™ thermal cycler” on page 6

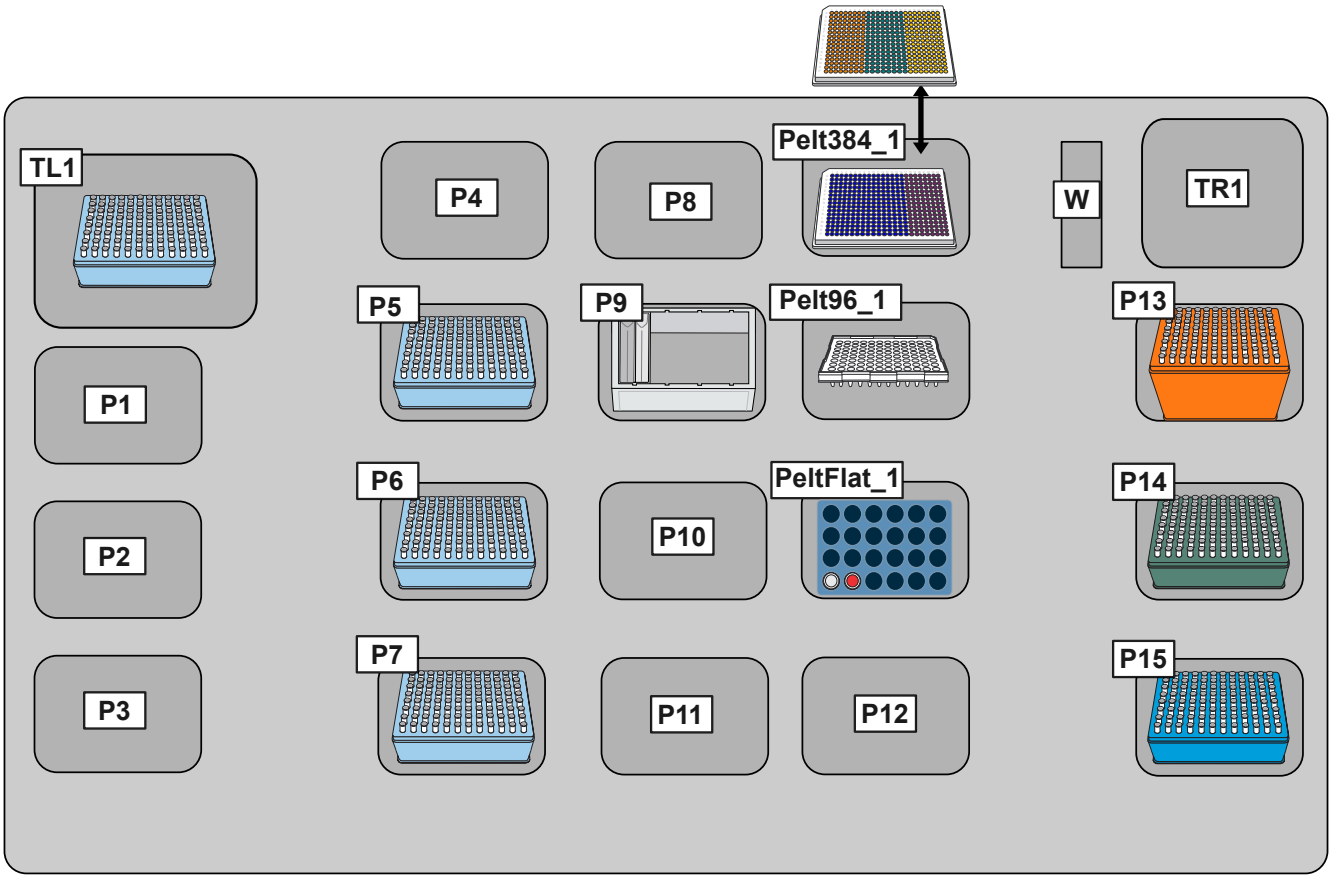


Fig. 3 Biomek™ FX^P deck setup for Ligation Reaction Setup Method

TL1	AP96 P20 Tips
P5	AP96 P20 Tips
P6	AP96 P20 Tips
P7	AP96 P20 Tips
P9	Ligation-qPCR Master Mix (quarter reservoir in frame, liquid on the left)
Pelt384_1	Plate 1/Plate 2
Pelt96_1	Fast 96-well plate
PeltFlat_1	0.5-mL rack (D1—DNA Ligase; D2—Master Mix Additive)
P13	Span-8 P1000 Tips
P14	Span-8 P250 Tips
P15	Span-8 P20 Tips

Run the ligation reaction on the ProFlex™ thermal cycler

Perform this procedure for Plate 1, then repeat for Plate 2.

1. Ensure that the heated cover setting is off.
2. Place the plate in the ProFlex™ thermal cycler, next to an empty 384-well EnduraPlate™.
3. Apply a compression pad over each plate, close the cover, then run the following program.

Temperature	Time
25°C	25 minutes
95°C	5 minutes
4°C	∞

4. Remove the plate from the thermal cycler, then place on ice or at 4°C for 5 minutes to ensure that the entire plate equilibrates to 4°C.
5. Tap the plate firmly on a benchtop 5–10 times to mix, centrifuge at 1,400 × *g* for 1 minute, then proceed to the next section.

Run the real-time PCR on the QuantStudio™ 12K Flex Instrument

1. In the QuantStudio™ 12K Flex Software, in the **Experiment** pane, click **Create**.
2. In the **Experiment Properties** screen, enter the experiment name, then select the experiment properties.

Property	Setting
Block	384-well
Experiment type	Standard Curve
Reagents	TaqMan™ Reagents
Instrument run	Fast

3. Click **Import** ▸ **Import Plate Setup**, navigate to, then select the plate setup file.
4. Click **Start Import**.
5. In the **Assign** screen, ensure that the assays and samples have been assigned to the appropriate wells.
6. In the **Run Method** screen, enter the thermal cycling parameters.
   - Reaction volume per well—20 µL
   -

Stage	Temperature	Time
Hold	95°C	20 seconds
PCR (40 cycles)	95°C	1 second
	60°C	20 seconds

7. Save the experiment (EDS file), then load the plate into the instrument.
8. Start the run.
9. After the run is complete, click **Analysis**.
10. In the **Analysis** screen, click **Analysis Settings**, ensure the following default C_t settings are selected, then click **Analyze** ▸ **Save**.
    - Baseline—Auto
    - Threshold—0.2
11. In **Experiment Menu**, click **Export**, then select the format, data, and location for export.
    - Data format—QuantStudio 12K Flex
    - File type—*.txt
    - Data—select the checkboxes in the **Sample Setup** and **Results** tabs.
12. Click **Start Export**.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0019000

Revision	Date	Description
A.0	27 February 2020	New document for new product launch.