Application Note:
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Pelleting Efficiency of Patented Tilted Adapters Using the New Thermo Scientific Benchtop 1-Liter Centrifuges

A Comparison of Pelleting in Thermo Scientific Benchtop and Superspeed Floor Model Centrifuges

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KEY WORDS
- Cellular Pelleting
- 1-Liter Benchtop Centrifuge
- Tilted Adapters
- Conical Tubes
- Swinging Bucket Rotor
- Superspeed Floor Model
- Fixed Angle Rotor
- Carbon Fiber Rotor

Introduction

Cellular pelleting is the initial step in many procedures in life science disciplines, including tissue culture, nucleic acid isolations, protein purifications, and subcellular fractionations. Fixed angle rotors and superspeed floor model centrifuges are typically utilized for large volume bacterial pelleting and subcellular fractionation studies whereas swinging bucket rotors and benchtop centrifuges are often used for smaller volumes in tissue culture applications and nucleic acid purifications.

The new Thermo Scientific general purpose benchtop centrifuges, with newly designed tilted conical tube adapters, maximize the capacity and separation efficiency in swinging bucket rotors. During rotation, centrifugal force is generated from the rotation axis outwards, in a radial direction (Figure 1).

Thermo Scientific Benchtop 1-Liter Centrifuge with TX-400 Swinging Bucket Rotor with 15 ml and 50 ml Adapters

Thermo Scientific Fiberlite F13-14x50 Rotor

15 ml Tilted Adapter

TX-200 Swinging Bucket Rotor with 15 ml and 50 ml Adapters

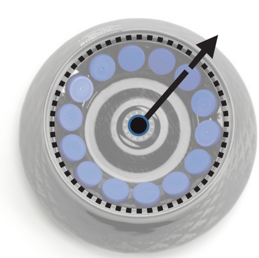

Figure 1. Radial force during centrifugation.

In a fixed angle rotor, as seen from the top, the tube cavities are aligned to the radial distribution of the relative centrifugal force (RCF). But due to the fixed angle configuration, the tube is not subjected to the RCF along a direct top-bottom direction. Alternatively, buckets in a swing-out rotor, apart from greater versatility, generally facilitate more favorable alignment. The buckets swing out to perfectly align with the centrifugal force (Figure 2).

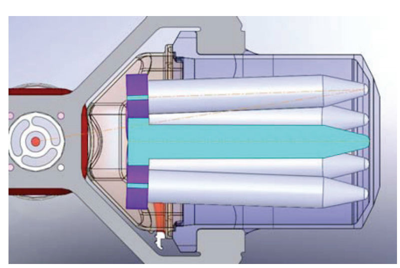

Figure 2. Force distribution in a swinging bucket rotor using tilted conical tube adapters.

However, upon greater examination, only the tubes positioned in the middle of the bucket, as seen from the top, are actually aligned with the RCF. The other tubes are all conventionally arranged in parallel to the middle tube, but the respective g-force is radial (and not parallel to the RCF of the tube in the middle). The radial g-force is applied with an angle which increases as the cavities get closer to the edge of the bucket. In this configuration, only a portion of the RCF works in a top-bottom direction. In fact, a minor portion pushes the tube on its side. This is generally accepted and usually does not influence the result of the separation. Nevertheless, the innovative design of the tilted Thermo Scientific conical tube adapters allows for the conical tubes to better align with the radial RCF, distributing the variation of the cavity angle in a very uniform way. As a result, not only is the capacity of the adapter improved, but also the quality of the separation.

The following application brief investigates the relative pelleting efficiency of benchtop versus floor model centrifuges, swinging bucket versus fixed angle rotors, and tilted versus non-tilted adapters. This brief describes sedimentation protocols for bacterial cells in medium (50 ml) and large (250, 400 ml) volume cultures in the new one liter general purpose benchtop centrifuge and compares the pelleting efficiency of carbon fiber rotors, such as the Thermo Scientific Fiberlite F13-14x50cy rotor, to metal, such as Thermo Scientific SLA-1500 or SS-34 superspeed fixed angle rotors. The pelleting efficiency and sample clarity was determined by measuring the absorbance of the supernatant.

Procedures

PROTOCOL 1:

Comparison of pelleting efficiency of large volume bacterial cultures in a benchtop swinging bucket rotor and superspeed fixed angle rotor

1. 175 ml of an overnight culture of *Escherichia coli (E. coli)* inoculated with a blank plasmid was placed into 400 ml polypropylene bio-bottles and also into 250 ml polypropylene standard Oak Ridge style bottles.
2. The 400 ml bottles were placed into a Thermo Scientific TX-400 swinging bucket rotor and the 250 ml bottles into the Thermo Scientific SLA-1500 rotor.
3. Run conditions were set as follows for each rotor/centrifuge combination, ensuring the bacterial sample experienced the same RCF:

a. TX-400 in benchtop 1L centrifuge: 4614 x g, 15 min, 4 °C

b. SLA-1500 in superspeed floor model: 4600 x g, 15 min, 4 °C

NOTE: Many protocols for bacterial pelleting in fixed angle superspeed floor model centrifuges use the following run conditions: 5000 x g, 10 min, 4 °C (Sambrook et al., 1989).

4. After centrifugation, the samples were removed from the rotor, and the absorbance of the supernatant was measured to determine sample clarity and pelleting efficiency.

Result

Conditions from the protocol above were run in triplicate and the average absorbance values were reported (Table 1).

ROTOR	RCF (XG)	TIME (MIN)	ABSORBANCE
TX-400	4614	15	0.13
SLA-1500	4600	15	0.019

Table 1. Comparison of pelleting efficiency of large volume bacterial cultures. Note: Color indicates clarity of absorbance value where green absorbance value is exceptional clarity, yellow is good clarity and red is average clarity.

Both of the pellets obtained from centrifugation in the fixed angle metal superspeed rotor (SLA-1500) and swinging bucket benchtop rotor (TX-400) were tightly packed and the supernatant was clear after spinning. The pellet positioning in the fixed angle rotor was distributed at an angle along the bottom wall of the bottle and the pellet in the swinging bucket was distributed along the bottom.

PROTOCOL 2:

Comparison of pelleting efficiency of medium volume bacterial cultures in benchtop swinging bucket, fixed angle benchtop and superspeed fixed angle rotors

1. 40 ml of an overnight culture of *Escherichia coli (E. coli)* inoculated with a blank plasmid was placed into 50 ml conical and also into 50 ml round bottom polypropylene tubes.
2. The 50 ml conical tubes were placed in the Thermo Scientific TX-400, TX-200, and Fiberlite F13S-14x50cy rotors and the 50 ml round bottom tubes were placed in the Thermo Scientific SS-34 rotor.
3. Run conditions were set as follows for each rotor/centrifuge combination, ensuring the bacterial sample experienced the same RCF:
   a. TX-400 with 50 ml conical adapter (PN 75003683) in benchtop 1L centrifuge: 4614 x g, 18 min, 4 °C
   b. TX-200 with 50 ml conical adapter (PN 75003803) in benchtop 1L centrifuge: 4614 x g, 18 min, 4 °C
   c. Fiberlite F13S-14x50cy in benchtop 1L centrifuge: 4,620 x g, 18 min, 4 °C
   d. Fiberlite F13S-14x50cy in benchtop 1L centrifuge: 12,460 x g, 6 min. 45 sec, 4 °C
   e. SS-34 in superspeed floor model: 4600 x g, 18 min, 4 °C

NOTE: The adapters used in the TX-400 and TX-200 rotor buckets are at a slight tilt.

4. After centrifugation, the samples were removed from the rotor, and the absorbance of the supernatant was measured to determine sample clarity and pelleting efficiency.

Result

Conditions from the protocol above were run in triplicate and the average absorbance values were reported (Table 2).

All pellets obtained from this set of experiments were tightly packed and in the appropriate place for the rotor and tube type. There was no smearing along the side walls. As demonstrated above, there is a comparable pelleting efficiency between all conditions whether the centrifugation was conducted in a fixed angle floor model rotor or a benchtop swinging bucket rotor. The pelleting efficiency (as determined by supernatant clarity) between the carbon fiber benchtop rotor (Fiberlite F13S-14x50cy) and the metal superspeed rotor (SS-34) are the exact same, suggesting that pelleting in a carbon fiber rotor in a benchtop centrifuge is just as efficient as pelleting conducted in a traditional metal rotor in a superspeed floor model centrifuge.

Lastly, when the g-force is increased to a speed of 12,460 x g in the carbon fiber rotor (Fiberlite F13S-14x50cy), the supernatant clarity among the samples is the clearest for all conditions. This offers the researcher a time savings of 11 min, 15 sec per run for an even more efficient pelleting.

ROTOR	ADAPTER	RCF (XG)	TIME (MIN:SEC)	ABSORBANCE
TX-400	75003683	4614	18:0	0.0103
TX-200	75003803	4614	18:0	0.0075
Fiberlite F13S-14x50cy	N/A	4620	18:0	0.006
Fiberlite F13S-14x50cy	N/A	12460	6:45	0.0045
SS-34	N/A	4600	18:0	0.006

Table 2. Comparison of pelleting efficiency of medium volume bacterial cultures.
Note: Color indicates clarity of absorbance value where green absorbance value is exceptional clarity, yellow is good clarity and red is average clarity.

PROTOCOL 3:

Comparison of pelleting efficiency of bacterial cultures in tilted and non-tilted benchtop swinging bucket adapters

1. 40 ml or 12 ml of an overnight culture of *Escherichia coli (E. coli)* inoculated with a blank plasmid was placed into 50 ml or 15 ml polypropylene conical tubes.
2. The conical tubes were then placed into the tilted and non-tilted adapters in the TX-400 and TX-200 rotors.
3. Run conditions were set as follows for each centrifuge/rotor combination to ensure the bacterial sample experienced the same g-force:
    a. TX-400 with tilted 15 ml adapter (PN 75003682) in benchtop 1L centrifuge: 4614 x g, 8 min, 4 °C
    b. TX-200 with tilted 15 ml adapter (PN 75003771) in benchtop 1L centrifuge: 4614 x g, 8 min, 4 °C
    c. TX-400 with non-tilted 15 ml adapter (PN 75007621) in benchtop 1L centrifuge: 4614 x g, 8 min, 4 °C
    d. TX-400 with tilted 50 ml adapter (PN 75003683) in benchtop 1L centrifuge: 4614 x g, 18 min, 4 °C
    e. TX-400 with non-tilted 50 ml adapter (PN 75007577) in benchtop 1L centrifuge: 4614 x g, 18 min, 4 °C
4. After centrifugation, the samples were removed from the rotor, and the absorbance of the supernatant was measured to determine sample clarity and pelleting efficiency.

Result

Conditions from the protocol above were run in triplicate and the average absorbance values were reported (Table 3).

As demonstrated in data table 3, the absorbance values of the supernatant is the lowest for all of the titled adapters regardless of volume. This suggests that **the patented tilted adapters offer the greatest pelleting efficiency.** All pellets obtained were tightly packed and supernatants clear for the centrifugation conditions.

Conclusion

The experiments above were conducted to examine the relative pelleting efficiencies of benchtop versus floor model centrifuges, swinging bucket versus fixed angle rotors, and tilted versus non-tilted adapters. Although bacterial pelleting is typically an application conducted in a superspeed floor model centrifuge, the results obtained from protocols 1 and 2 indicate that comparable pelleting efficiencies can be achieved when using a benchtop centrifuge (Table 4).

In the absence of a floor model centrifuge and associated accessories, the new general purpose benchtop centrifuge with swinging bucket rotor or carbon fiber fixed angle rotor can also be used for bacterial pelleting. This highlights the versatility of the new general purpose centrifuge and despite the large difference in K-factors between swinging bucket and fixed angle rotors, the same applications can be done using both equipment sets and comparable protocol conditions.

It is also important to note that bacterial pelleting conducted in a 50 ml conical in the Fiberlite carbon fiber benchtop rotor at high g-force (~12,460 x g) achieves very high sample clarity and can offer the researcher valuable time savings by decreasing the centrifugation time by 11 min, 15 sec per run. This allows the researcher to focus more on achieving results and less on protocol.

Lastly, the newly designed Thermo Scientific **tilted adapters offer sample clarity greater than those achieved with traditional non-titled adapters.** This unique, patented design, when combined with the new general purpose centrifuges, presents the best tools on the market for the most efficient research.

In conclusion, whether pelleting bacteria for nucleic acid isolations or protein purification in a superspeed floor model or benchtop centrifuge, be assured that with the appropriate accessory and rotor combination, efficient results can be obtained with Thermo Scientific products.

TILTED ADAPTERS PROVIDES EXCEPTIONAL SAMPLE CLARITY COMPARED TO NON-TILTED ADAPTERS
FIBERLITE CARBON FIBER ROTORS IN A BENCHTOP CENTRIFUGE PROVIDE EXCELLENT SAMPLE CLARITY AND REDUCED CENTRIFUGATION TIME
PELLETING IS EFFICIENT IN MULTIPLE THERMO SCIENTIFIC CENTRIFUGATION PRODUCTS

Table 4. Conclusions of pelleting efficiency comparisons.

Reference

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular cloning: A laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

ROTOR	ADAPTER	VOLUME	TILTED	RCF (XG)	TIME (MIN)	ABSORBANCE
TX-400	75003682	15	**Yes**	4614	8	**0.004**
TX-200	75003771	15	**Yes**	4614	8	**0.00775**
TX-400	75007621	15	No	4614	8	**0.02**
TX-400	75003683	50	**Yes**	4614	18	**0.0103**
TX-400	75007577	50	No	4614	18	**0.231**

Table 3. Comparison of pelleting efficiency of bacterial cultures in tilted and non-tilted adapters.
Note: Color indicates clarity of absorbance value where green absorbance value is exceptional clarity, yellow is good clarity and red is average clarity.

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